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Studies on the Anatomy of *Rhamnus lanceolata* Pursh and *Rhamnus Frangula* L.*

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This investigation was started in order that the anatomy of the genus *Rhamnus* of the family *Rhamnaceæ* might be better understood. The authors searched the literature for information relative to the anatomy of this genus and found that such information was relatively scant excepting for the barks of a few species. Solereder (1) in his "Systematic Anatomy of the Dicotyledons" offered a small amount of general information. Gathercoal (2) in a paper on the medicinal *Rhamnus* barks described the microscopy of a few of these. Rusby (3) and Sayre (4) have also contributed histological data on the barks of several *Rhamnus* species. Literature on the general anatomy of *Rhamnus lanceolata* Pursh is entirely lacking.

Materials and Methods.—The material examined was gathered in October, 1938, at the Arnold Arboretum, Jamaica Plain, Mass., from properly identified plants. Most of the material was immediately preserved in equal parts of glycerin and alcohol. From this preserved material portions of the stem were dehydrated in a series of alcohols and imbedded in celloidin. From this transverse, tangential-longitudinal and radial-longitudinal sections of both species were cut on the sliding microtone and stained with fast green and safranin and then mounted in Canada balsam. Some of the sections were mounted unstained in glycerin jelly. Others were treated with phloroglucin-hydrochloric acid and mounted in glycerin jelly. This was done to ascertain the amount of lignification in various tissues. Leaf and petiole material was killed and fixed with chrome-acetic fixative, dehydrated by the butyl alcohol method, imbedded in paraffin, cut on both rotary and sliding microtones and stained in fast green and safranin.

The scope of this work includes the anatomy of the stem, leaf and petiole of *Rhamnus lanceolata* Pursh and *Rhamnus Frangula* L. Some work was also done on the anthraquinone contents of the barks of these 2 species.

DESCRIPTION OF THE PLANTS

Rhamnus lanceolata Pursh.—This plant is a shrub 1 to 2 M. tall with smooth, grayish bark; leaf blade firm, oblong or oblong lanceolate or sometimes oval, acute or acuminate, serrulate, abruptly acute or rounded at the base, short petioled, glabrous or minutely downy and light green beneath with 7 to 9 pairs of veins. Flowers of 2 forms on distinct plants, both perfect; one with short pedicels clustered and with a short included style; the other with pedicels oftener solitary, style exserted; petals deeply

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notched. The fruit is globular, 5 to 7 mm. thick, 2-seeded, the seeds furrowed on the back.

The plant is found on river banks and moist hillsides from Pennsylvania to Iowa, Alabama, Texas and Colorado.

Rhamnus Frangula L.—This plant is a shrub or small tree up to 6 M.; young branchlets pubescent; leaves oval or obovate to obovate-oblong, 3–7 cm. long, acute, rounded or broad-cuneate at base, entire, dark lustrous green above, lighter green and often slightly pubescent beneath with 8 or 9 pairs of veins; petiole 6 to 12 cm. long; flowers in clusters of 2 to 10, glabrous; pedicels 8 to 12 mm. long; fruit globose, 6 mm. across, changing from red to dark purple, 2-seeded.

The plant is native to Europe, Western Asia and Northern Africa but is naturalized in the Eastern states and also in Illinois.

ANATOMY OF *Rhamnus lanceolata* PURSH

Transverse Section of the Stem of Rhamnus lanceolata Pursh.—In transverse section (Plate I, Figs. 1 and 3), a 2-yr.-old stem shows a phellem which averages 20 cells wide, consisting of flat, tubular cells arranged in radial rows. The outer 6 to 8 rows of cells are much compressed and highly suberized with lumina reduced to thin lines. The inner cells are much larger and each shows a distinct lumen. These cells average 35 μ in length and 9 μ in breadth with cell walls showing an average thickness of 3 μ. Lenticels are numerous.

The phellogen occurs as a single layer of thin-walled cells of about the same size as the phelloderm cells but having thin walls and being rich in proplasmic content.

The phelloderm in transverse view consists of a narrow zone of cells immediately below the phellogen. This zone is from 4 to 5 cells in width. The cells are somewhat collenchymatous with walls which average 3 μ thick in the angles and 1.5 μ thick elsewhere. The cells of the phelloderm are arranged in slightly irregular radial rows.

The middle bark, comprising the cortex and pericycle (since these zones are not clearly differentiated), consists of a broad zone of cells ranging from 15 to 25 cells in width. These cells are also collenchymatous in the outer regions, but passing inward, the cells gradually lose their collenchymatous character until the inner portion of the cortex consists of ordinary parenchyma. Cortex cells are usually slightly larger than the phelloderm cells and tend to be oval in outline. In the inner portion of the middle bark, there are found groups of sclerenchyma fibers arranged in an interrupted circle. The groups contain usually 15 to 20 fibers. These fibers are very thick walled in transverse section, the lumina being reduced to very thin lines. The lignification of these fibers varies with the age of the stem, lignification being more pronounced in older stems. The sclerenchyma fibers of the middle bark are often partially surrounded by cells bearing monoclinic prisms of calcium oxalate. The middle bark also shows many cells containing a single rosette aggregate of calcium oxalate which takes up practically the entire lumen of the cell.

The phloem, as viewed in transverse section, is seen to consist largely of thin-walled, rectangular phloem parenchyma with cells averaging 11 μ in length and 6 μ in breadth. The phloem in the 4-yr.-old stem contains many small groups of phloem fibers consisting usually of 10 fibers. As seen in transverse section, these fibers are oval to compressed and have thick walls as do the cortical fibers. Here, too, the fibers are surrounded by cells bearing a monoclinic prism of calcium oxalate. Phloem rays are distinguished from the phloem parenchyma with greatest difficulty. In most cases phloem rays were 2 to 3 cells wide and closely resembled phloem parenchyma. Rosette aggregates are scattered throughout the phloem.

The cambium consists of a single layer of flattened, rectangular, thin-walled cells only slightly smaller than the phloem parenchyma.

The xylem is 5 to 6 times the width of the bark and is made up entirely of vessels, wood rays and wood fibers. Wood parenchyma and tracheids appear to be entirely wanting. The wood is heterogeneous and distinctly diffuse porous. Vessels occur both singly and in groups of 15 to 20. The vessels of the late wood are somewhat smaller than those of the early wood which run up to 40 μ in diameter. The fibers which occupy most of the wood occur in radial rows and are thin walled, rectangular to pentangular and average 12 μ in diameter. The fibers of the late wood are of about the same size as those of the early wood but differ in being thicker walled. These walls average 3 μ. Wood rays consist of rectangular cells, averaging 3.5 μ in breadth and 17 μ in length. The rays in transverse section are from 1 to 3 cells wide. The cells are thin-walled and rich in protoplasmic content.

The pith is surrounded by a small amount of primary wood and consists of very large parenchyma cells, bearing occasionally, a rosette aggregate. These crystals average 22 μ in diameter.

DESCRIPTION OF PLATE I

Fig. 1.—T. S. of *Rhamnus lanceolata* Pursh showing various regions of the bark: (*A*) and (*B*) phellem, (*C*) phellogen, (*D*) phelloderm, (*E*) cortex, (*F*) rosette, (*G*) sclerenchyma fibers, (*H*) phloem parenchyma, (*I*) phloem fibers.

Fig. 2.—T. S. *Rhamnus Frangula* L. showing portions of the bark: (*A*) phellem, (*B*) phellogen, (*C*) phelloderm, (*D*) cortex, (*E*) pericyclic fibers, (*F*) phloem ray, (*G*) phloem.

Fig. 3.—T. S. *Rhamnus lanceolata* Pursh showing a portion of the phloem and wood: (*A*) rosette, (*B*) phloem, (*C*) monoclinic prisms, (*D*) phloem ray, (*E*) cambium, (*F*) xylem, (*G*) wood fiber, (*H*) wood ray, (*I*) vessel, (*J*) xylem, (*K*) pith.

Fig. 4.—T. S. of *Rhamnus Frangula* L. showing portion of phloem and xylem (wood): (*A*) phloem fiber, (*B*) phloem ray, (*C*) phloem parenchyma, (*D*) cambium, (*E*) xylem, (*F*) wood fiber, (*G*) vessel, (*H*) wood parenchyma, (*I*) wood ray, (*J*) xylem, (*K*) vessel, (*L*) wood fiber, (*M*) pith.

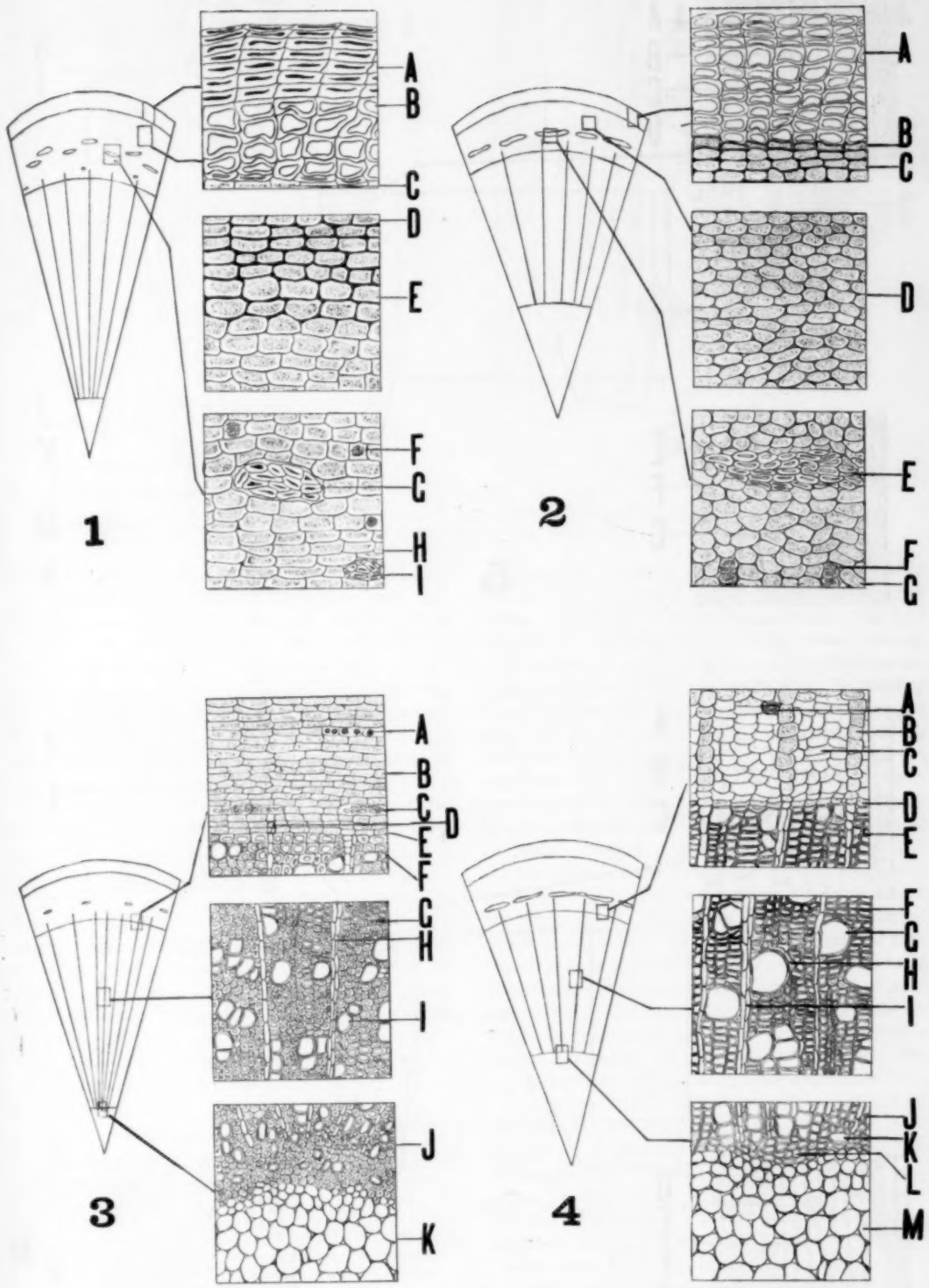

PLATE I
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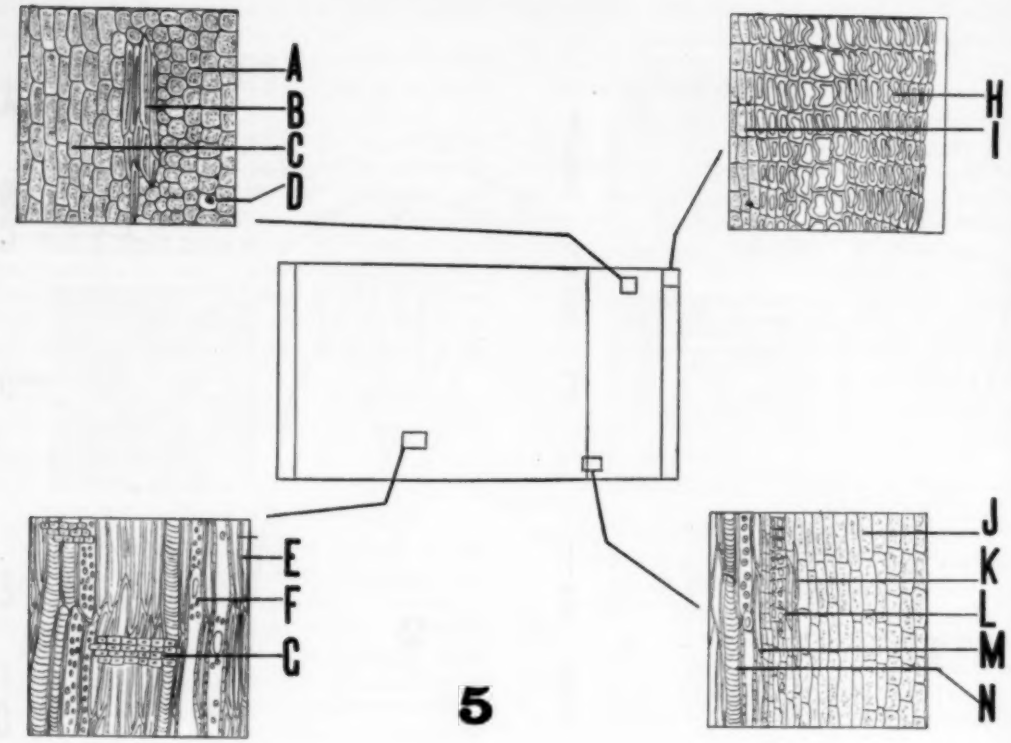

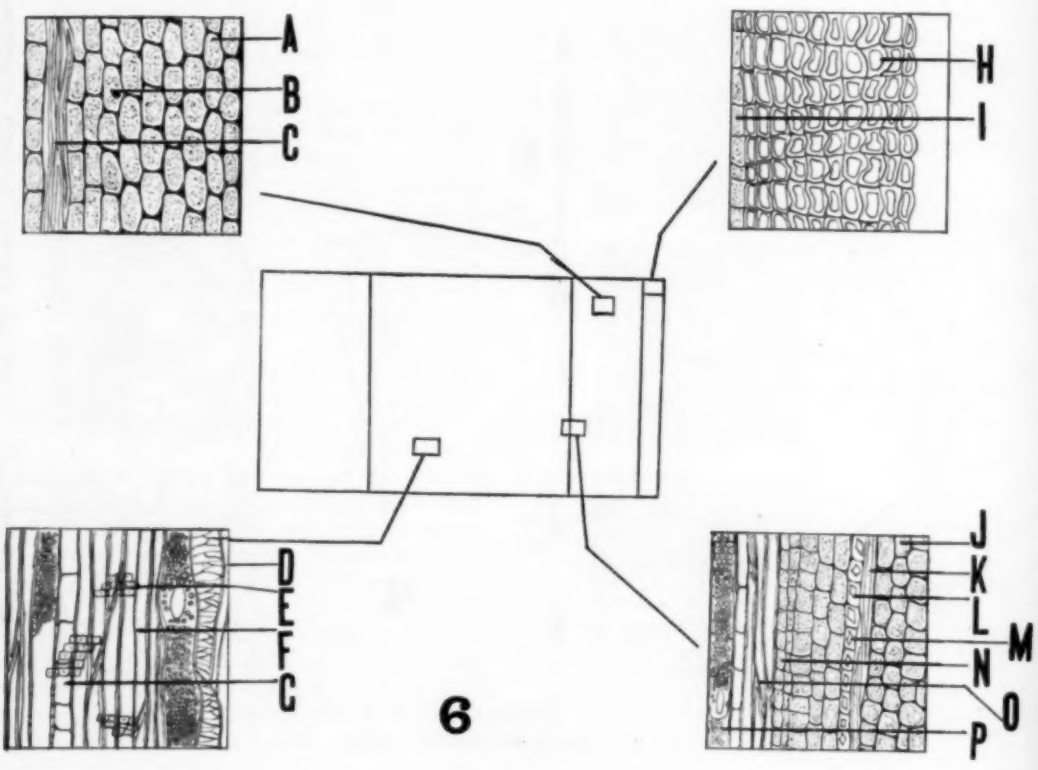

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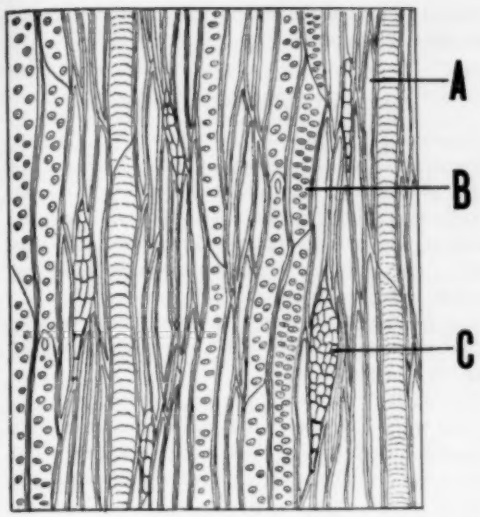

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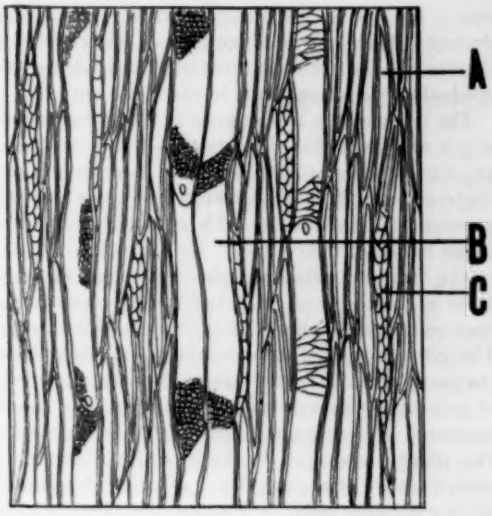

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PLATE III

Fig. 7.—T. L. S. of the wood of *Rhamnus lanceolata* Pursh: (*A*) wood fiber, (*B*) vessel, (*C*) wood ray.
Fig. 8.—T. L. S. of the wood of *Rhamnus Frangula* L.: (*A*) wood fiber, (*B*) vessel, (*C*) wood ray.

Longitudinal Section of the Stem of Rhamnus lanceolata Pursh.—In radial-longitudinal and tangential-longitudinal views (Plates II and III, Figs. 5 and 7), the phellem, phellogen and phelloderm appear much the same as in transverse view. In the cortex, however, the cells in the outer zone consisting of collenchyma appear somewhat more cubical than in the transverse view. The sclerenchyma fibers of the cortex are found to be narrow and vertically elongated. These fibers are commonly 300 μ long. They average 15 μ wide, the cell wall being about 3.5 μ thick. The crystal fibers consist of long vertical rows of rectangular cells vertically elongated, each bearing a monoclinic prism of calcium oxalate.

The phloem parenchyma cells offer no differentiation in longitudinal views. The phloem fibers are usually somewhat smaller than the cortical fibers but are more heavily lignified. Crystal fibers, as described above, also accompany the phloem fibers. They are more numerous with phloem fibers than with the cortical fibers. The sieve tubes are vertically elongated, rectangular and have callous plates on both the vertical and transverse walls.

DESCRIPTION OF PLATE II

Fig. 5.—R. L. S. of *Rhamnus lanceolata* Pursh showing portions of bark and wood: (*A*) cortex, (*B*) pericyclic fibers, (*C*) phloem, (*D*) rosette, (*E*) wood fiber, (*F*) vessel, (*G*) wood ray, (*H*) phellem, (*I*) phellogen, (*J*) phloem parenchyma, (*K*) pericyclic fiber, (*L*) crystal fiber, (*M*) cambium, (*N*) vessel.

Fig. 6.—R. L. S. of *Rhamnus Frangula* L. showing portions of the bark and wood: (*A*) phelloderm, (*B*) cortex, (*C*) pericyclic fiber, (*D*) vessel, (*E*) wood ray, (*F*) wood fiber, (*G*) wood parenchyma, (*H*) phelloderm, (*I*) phellogen, (*J*) phloem parenchyma, (*K*) phloem fiber, (*L*) crystal fiber, (*M*) monoclinic prism, (*N*) cambium, (*O*) wood fiber, (*P*) vessel.

The vessels of the wood for the most part are of the bordered-pore type. These pores are scattered rather irregularly over the vessel wall. The end wall of the vessel exhibits a simple pore. Vessels with scalariform and reticulate wall thickenings are occasionally found but are much fewer in number than the vessels showing bordered pores. The wood rays are prominent in radial-longitudinal section and exhibit 2 distinct parts, the ray proper and the marginal areas. The cells of the ray proper are rectangular and elongated in a horizontal plane, averaging 25 μ in length and 12 μ in breadth. The marginal ray cells, however, appear more nearly square in outline although the outermost wall is frequently irregular. These cells average 25 μ square. Both marginal ray cells and cells of the ray proper exhibit simple pitted walls. In tangential-longitudinal view the wood rays are from 1 to 3 cells wide and up to 19 cells long. The average ray, however, is 2 cells wide and 13 cells long. In this plane the cells are vertically elongated, rectangular to slightly ovoid in outline, averaging 17 μ tall and 10 μ wide. The wood fibers in longitudinal view are commonly 300 μ long and 14 μ wide, usually more or less lignified dependent upon the age of the stem.

Anatomy of the Leaf of Rhamnus lanceolata. A: Transverse Section of the Petiole.—In transverse view (Plate IV, Fig. 11) the outline of the petiole is orbicular to slightly oval. The epidermis consists of isodiametric cells averaging 17 μ in diameter, bearing a cuticle which is on the average 5 μ thick.

Directly beneath the epidermis on the dorsal side of the petiole is an area of supporting collenchymatous cells. This area is 5 cells wide in the mid-dorsal area and gradually narrows, passing around the petiole until it is absent beneath the epidermis on the ventral

side. These cells are slightly larger than the epidermal cells and are oval in outline. The collenchymatous zone of the outer area of the petiole merges gradually into parenchyma in the inner cortex.

The inner cortex is composed of parenchyma cells which are up to 30 μ in diameter. Rosette aggregates are numerous in this area. The largest one observed was 28 μ in diameter, but these crystals average 20 μ in diameter. They usually occupy the entire lumen of the cell.

The vascular tissue assumes a crescent arrangement in the center of the petiole and is composed of open collateral bundles separated by medullary rays. The xylem is toward the ventral side and the phloem the dorsal side. The phloem patches consist largely of very small parenchymatous cells and sieve tubes, separated by somewhat larger phloem ray cells. The phloem averages 7 cells in width. The cambium consists of a single layer of small thin-walled rapidly dividing cells.

The xylem patches are 2 to 5 cells wide and average 12 cells long. The patches consist of wood fibers with thickened walls and vessels which average 17 μ in diameter. Both fibers and vessels are lignified. The xylem patches are separated by wood rays that are 1 to 2 cells wide.

B: Epidermises of the Leaf.—The cells of the upper epidermis are of 2 distinct types. Those cells which are located over the veins are rectangular and have their long axes running parallel to the veins. The other cells of the epidermis are irregularly polygonal. These cells average 30 μ at their greatest diameter and 17 μ at their narrowest diameter.

The lower epidermal cells situated directly beneath the veins are elongated in the same manner as those of the upper epidermis. The other cells are irregularly rectangular and average 24 μ by 16 μ. Many stomata are present. The guard cells are long, narrow and sausage-shaped, averaging 23 μ long and 7 μ wide. Each stomate is surrounded by 4 neighboring cells.

C: Transverse Section of the Leaf Blade.—The transverse section (Plate IV, Fig. 9) shows the epidermis to consist of a layer of rectangular cells which average 17 μ in length and 10 μ in vertical width and bearing a cuticle which averages 3 μ thick. Directly below the upper epidermis is a double layer of palisade parenchyma occupying three-fourths of the area of the entire mesophyll. The cells of the outer layer of palisade are considerably larger than those of the inner layer, averaging 50 μ in height and 14 μ wide. The cells of the inner layer average 25 μ in height and 14 μ wide. The remainder of the mesophyll consists of ordinary spongy parenchyma. Chloroplasts are equally abundant throughout the mesophyll. The lower epidermis consists of more or less square-shaped cells which average 17 μ in diameter. Stomata are numerous with pear-shaped guard cells which are set diagonally against the end walls of the neighboring cells.

The structure of the midrib, in general, is identical with that of the petiole.

ANATOMY OF *Rhamnus Frangula* L.

Transverse Section of the Stem of Rhamnus Frangula L.—In transverse view (Plate I, Figs. 2 and 4) the phellem of a 3-yr.-old stem consists of an area of cells averaging 10 cells wide. The cells are rectangular, somewhat compressed and have suberized walls. The cells are arranged in radial rows and average 13.6 μ in length and 6.5 μ in breadth.

The phellogen consists of a single layer of thin-walled rectangular cells which are slightly smaller than the phellem cells.

The phelloderm, when present, comprises a zone of thin-walled to somewhat collenchymatous cells arranged in radial rows beneath the phellogen. This area is usually 3 cells wide with the individual cells slightly larger than the phellogen cells. Very frequently the phelloderm is absent both in younger and older stems.

The cortex consists of a zone, 12 to 15 cells in width. The cells of the outer region of the cortex are oval in outline and definitely collenchymatous. The cells of the inner portions of the cortex are parenchymatous and thin walled and somewhat less distinctly oval. The cells of the cortex vary considerably in size although the average cell is 34 μ in length and 13 μ in breadth.

Immediately beneath the cortex and outside of the phloem there occurs an interrupted circle of groups of pericyclic fibers. Frequently, these groups of fibers extend tangentially in the pericycle 12 to 15 cells. These groups, however, are rarely over 3 to 4 cells wide. Each fiber is very thick-walled, irregularly oval to almost polygonal in outline, and averages 25 μ in length and 10 μ in breadth. The lumen in most cases is very much reduced. Although the walls of the fibers are thick, they are not always lignified especially in younger stems. As the stem becomes older, the lignification becomes more pronounced.

The phloem in transverse view is 8 to 10 cells wide and composed largely of somewhat rectangular, thin-walled, phloem parenchyma cells that average 17 μ in length and 12 μ in breadth. Sieve tubes are not easily or surely defined in transverse section. Phloem rays, however, were quite prominent. The cells are arranged in radial rows and are more nearly square than the phloem parenchyma. Phloem fibers, though absent in younger stems, are present in older stems. Groups of up to 25 fibers are often seen. In transverse section the fibers are rectangular to pentangular with extremely thickened lignified walls, the lumen appearing as a faint line. Occasionally a few crystal fibers bearing a prism of calcium oxalate are seen accompanying these groups of fibers.

The cambium in transverse section appears as a single layer of small, thin-walled, rectangular cells approximating the size of the smaller phloem cells.

The xylem of the 3-yr.-old stem is about 3 times the width of the bark zone. It is definitely diffuse

porous, the vessels occurring scattered singly and in groups of up to 5 vessels. The xylem consists mostly of wood fibers, vessels and wood rays. Wood parenchyma is present in small amounts. The wood fibers in transverse section are usually rectangular and arranged in radial rows that extend the width of the xylem. They are for the most part thin-walled, but in the areas of the late wood the cell walls are somewhat thickened. The wood fibers in transverse section average 15 μ in length and 10 μ in breadth, and are lignified in varying degrees in different regions of the stem. The vessels vary from circular to polygonal and average 40 μ in diameter. Accompanying the vessels occasional wood parenchyma cells are seen. They are, however, relatively scant. The wood rays in transverse section are 1 to 2 cells wide, very rarely up to 3 cells wide. The individual cells are rectangular and radially elongated, the average cell being 25 μ in length and 11 μ in width. The innermost part of the wood consists of a small amount of primary xylem.

The pith is, in this species, rather large. In a stem 5 mm. in diameter, the pith is about 2.5 mm. in diameter. It is composed of very large ordinary parenchyma cells which average 100 μ in diameter.

Longitudinal Sections of the Stem of Rhamnus Frangula L.—In longitudinal section (Plates II and III, Figs. 6 and 8) the phellem, phellogen and phelloderm are much the same as when viewed in transverse section. The collenchymatous region of the cortex is also as it is in transverse section. The inner or parenchymatous portion of the cortex is somewhat different in that cells appear a little more square.

The pericyclic fibers in longitudinal section are extremely long and thick-walled, the lumen appearing as a thin line running the length of the fiber. Frequently these fibers attain a length of 200 μ and have tapering end walls.

The phloem parenchyma is mostly rectangular and occupies the greater portion of the phloem. Phloem rays are easily distinguished in radial longitudinal view, as radial rows of square cells, these cells running at right angles to the phloem parenchyma. In tangential-longitudinal view, the phloem rays are seen to be 2 to 3 cells wide and 15 cells tall vertically. The cells are rather square and average 15 μ in diameter. Sieve tubes are occasionally seen arranged in vertical rows, each tube being up to 135 μ long and 30 μ wide. They are usually accompanied by companion cells which are a little narrower and somewhat longer than the tube itself. In longitudinal view the phloem fibers are very much like the cortical fibers except that they are somewhat smaller and more deeply lignified. Occasionally a crystal fiber is seen accompanying the phloem fiber. Each cell of the fiber contains a monoclinic prism of calcium oxalate.

In longitudinal view the vessels are seen to be of 3 types, namely, bordered pored, scalariform and reticulate. The walls of the vessels with bordered pores are very densely packed with these pores. This type of vessel is much more abundant than the other 2 types. The wood parenchyma which occasionally accompanies the vessels is seen to be composed of vertical rows of vertically elongated, rectangular, thin-walled cells. The cells average 30 μ long and 9 μ wide. The wood fibers, which make up the bulk of the xylem, are extremely vertically elongated with very gradually tapering ends. These fibers are up to 500 μ long and 14 μ wide. The wood rays in radial longitudinal view consist of 2 types of cells, namely, those of the marginal area and those of the ray proper. The cells of the marginal area are square and average 28 μ in diameter. The cells of the ray proper are horizontally elongated and average 27 μ long and 17 μ wide. In tangential view the wood rays are up to 25 cells in vertical depth and up to 3 cells wide. More commonly, however, the rays are 1 to 2 cells wide and 15 cells in depth. The individual cells are irregularly rectangular and vertically elongated. They average 17 μ in length and 8 μ in width.

The pith merely consists of very large ordinary parenchyma cells.

Anatomy of the Leaf of Rhamnus Frangula L. *A: Transverse Section of the Petiole.*—The petiole in transverse view (Plate IV, Fig. 12) is more or less orbicular. The cells making up the epidermis are small, oval to ellipsoidal and bear a thick cuticle. Directly beneath the epidermis in the dorsal portion of the leaf is a zone of somewhat thick-walled supportive collenchyma. This zone is 4 cells wide in the mid-dorsal portion but gradually becomes narrower in passing around the petiole until it is entirely wanting on the ventral side. Beneath the supportive tissue is a rather wide zone of ordinary parenchyma averaging 20 cells wide.

In this area on the dorsal side schizogenous reservoirs occur in an interrupted circle throughout the center of the cortex. These reservoirs are oval to circular, up to 140 μ in diameter and surrounded by 8 to 10 crescent-shaped to oval cells. The vascular tissue is of the open collateral type and is arranged in a crescent formation with phloem on the outer dorsal side and xylem on the inner ventral side. The phloem patches merely consist of small groups of small phloem parenchyma cells and sieve tubes. The phloem patches are separated by phloem rays which are 1 to 2 cells in width. Ventral to the phloem patches and beneath the cambium are the xylem patches. These consist of groups of up to 15 vessels accompanied by wood fibers and wood parenchyma. The xylem patches are separated by wood rays which are 1 to 2 cells wide. The individual ray cells are isodiametric.

B. Epidermises of the Leaf.—The upper epidermis shows two different types of cells in surface view. The cells occurring over the veins are rectangular and have their long axes running parallel with the veins. The other cells of the upper epidermis vary in shape from rectangular to polygonal and average 35 μ in diameter. Nonglandular, unicellular, slightly curved hairs frequently occurring in pairs, are occa-

sionally found on that part of the epidermis directly over the midrib and larger veins. These hairs are thick walled, have a narrow lumen, and average 100 μ in length.

The lower epidermis also shows 2 types of cells as described above. However, the cells beneath the midrib and veins are smaller than the corresponding cells of the upper epidermis. The other cells of the lower epidermis vary from rectangular to polygonal in outline. The lower epidermis shows numerous stomata, the guard cells of which average 20 μ long and 10 μ wide. The stomata have 5 to 6 neighboring cells.

Transverse Section of the Leaf Blade.—The leaf is typically dorsiventral. In transverse view (Plate IV, Fig. 10) the upper epidermis is seen to be composed of rectangular cells averaging 25 μ in length and 17 μ in breadth. Beneath the upper epidermis is a single row of palisade parenchyma that occupies about one-third of the mesophyll. These cells are somewhat broader beneath the epidermis and taper toward the spongy parenchyma and average 17 μ in length and 8 μ in width. Beneath the palisade is a rather broad zone of typical spongy parenchyma. Chloroplasts appear to be equally abundant in palisade and spongy parenchyma. The lower epidermal cells are rectangular and about two-thirds the size of the upper epidermal cells. Stomata are frequently seen in the transverse view of the leaf. The guard cells of the stomata are rather small, irregularly oval. They are placed at about a 45° angle to the end walls of the neighboring cells. The histology of the midrib approximates that of the petiole.

COMPARISON OF THE ANATOMY OF THE BARKS OF *Rhamnus Frangula* L., *Rhamnus lanceolata* PURSH AND *Rhamnus Purshiana* DE CANDOLLE

The phellem of the 3 species is very similar, all being composed of rectangular cells with suberized walls. The phellogen also shows little differentiation in the 3 species.

The phelloderm of *Rhamnus lanceolata* is very similar to that of *Rhamnus Purshiana* but is not as collenchymatous as the latter. In *Rhamnus Frangula* L. the phelloderm is often lacking, but when present resembles that of *R. lanceolata*.

The outer cortex in all three species is very similar. In each case it consists of a narrow zone of oval collenchymatous cells.

The inner cortex of the three species is composed of ordinary parenchyma. However, in *R. lanceolata* and *R. Purshiana* there are many groups of cortical fibers which are associated with crystal fibers bearing monoclinic prisms of calcium oxalate. The groups of fibers in *R. lanceolata* contain on the average fewer cells than the groups of fibers in *R. Purshiana*.

Although *R. Frangula* L. has no cortical fibers, it does show a definite ring of groups of fibers in the pericycle.

The phloem of both *R. lanceolata* and *R. Purshiana* contains many groups of lignified phloem fibers associated with crystal fibers. It also contains many rosette aggregates, and sieve tubes with callous plates on the longitudinal and transverse walls. The phloem rays of the *R. Purshiana*, however, are much more clearly defined than those of *R. lanceolata*, the phloem rays of the latter being composed of cells which resemble, to a great degree, the phloem parenchyma. *R. Frangula* L. also shows groups of very thick-walled phloem fibers and, although these are occasionally accompanied by crystal fibers, neither the phloem fibers nor the crystal fibers are as abundant as in *R. lanceolata* and *R. Purshiana*. Rosette aggregates are absent in the phloem of *R. Frangula* L. The phloem rays are clearly defined and are 1 to 3 cells in width.

It may be said that *R. lanceolata* and *R. Purshiana* are quite similar in respect to the anatomy of their barks, while *R. Frangula* L. differs in several respects as was indicated in the foregoing comparison. These differences are mainly: the absence of cortical fibers, the presence of pericyclic fibers and a smaller amount of crystal fibers accompanying the phloem fibers.

A COMPARISON OF THE ANTHRAQUINONE CONTENT OF *Rhamnus lanceolata* PURSH AND *Rhamnus Frangula* L. WITH THAT OF *Rhamnus Purshiana* DC.

Believing that a comparison of the anthraquinone content of these 2 species with that of *Rhamnus Purshiana* (Cascara Sagrada) might be of pharmacognostic import, experiments were carried out.

Portions of both barks when treated on their inner face with 5% sodium hydroxide solution, gradually developed a deep red color.

The identity tests for Cascara Sagrada, as described in the U. S. P. XI, were then carried out on the 2 species. These tests consisted of adding, in each case, 0.1 Gm. of the powdered bark to 10 cc. of hot water, shaking the mixtures until cold, filtering and then bringing the volumes up to 10 cc. and adding 10 cc. of ammonia T. S. The resulting colors were in both cases deep red with a tinge of orange. This color was in both cases more intense than the color produced by a sample of Cascara Sagrada which was run at the same time.

One-tenth gram of powdered barks of both species was macerated with 10 drops of alcohol and 10 cc. of water. The mixtures were boiled, cooled and filtered. The filtrates were shaken with 10 cc. of ether; thereupon a yellow ethereal solution separated out. Three cubic centimeters of the ethereal solutions were shaken with 3 cc. of ammonia T. S. The

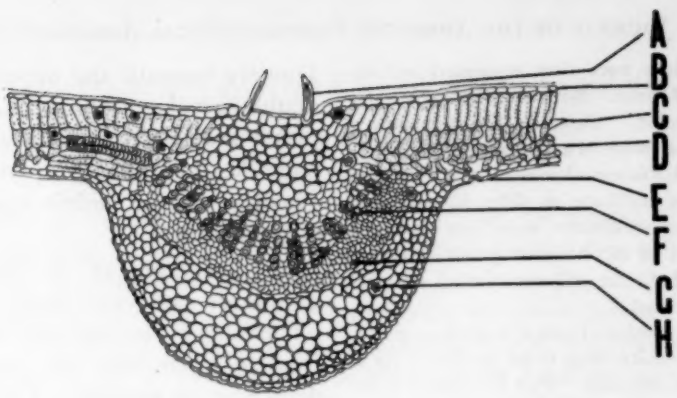
DESCRIPTION OF PLATE IV

Fig. 9.—T. S. of leaf of *Rhamnus lanceolata* Pursh: (*A*) hair, (*B*) upper epidermis, (*C*) palisade, (*D*) spongy mesophyll, (*E*) stomate, (*F*) xylem, (*G*) phloem, (*H*) rosette.

Fig. 10.—T. S. of the leaf of *Rhamnus Frangula* L.: (*A*) upper epidermis, (*B*) palisade, (*C*) spongy mesophyll, (*D*) lower epidermis, (*E*) stomate, (*F*) xylem, (*G*) cambium, (*H*) phloem, (*I*) mesophyll, (*J*) schizogenous secretory reservoir.

Fig. 11.—T. S. of the petiole of *Rhamnus lanceolata* Pursh: (*A*) upper epidermis, (*B*) mesophyll, (*C*) rosette, (*D*) xylem, (*E*) cambium, (*F*) phloem, (*G*) lower epidermis.

Fig. 12.—T. S. of the petiole of *Rhamnus Frangula* L.: (*A*) upper epidermis, (*B*) mesophyll, (*C*) xylem, (*D*) cambium, (*E*) phloem, (*F*) mesophyll, (*G*) schizogenous secretory reservoir, (*H*) lower epidermis.



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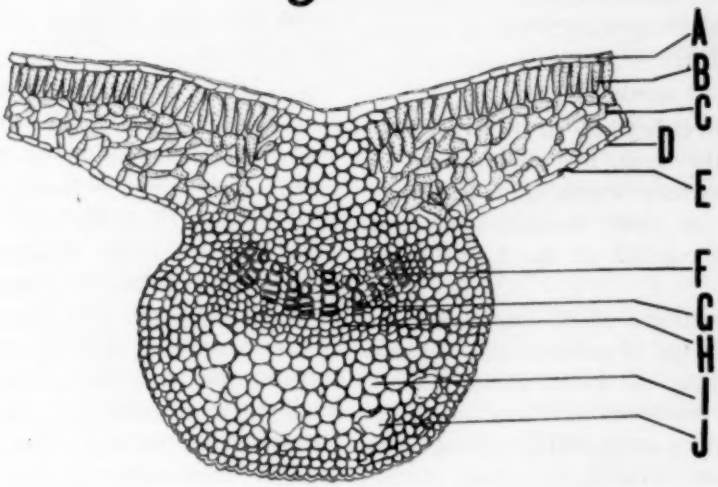

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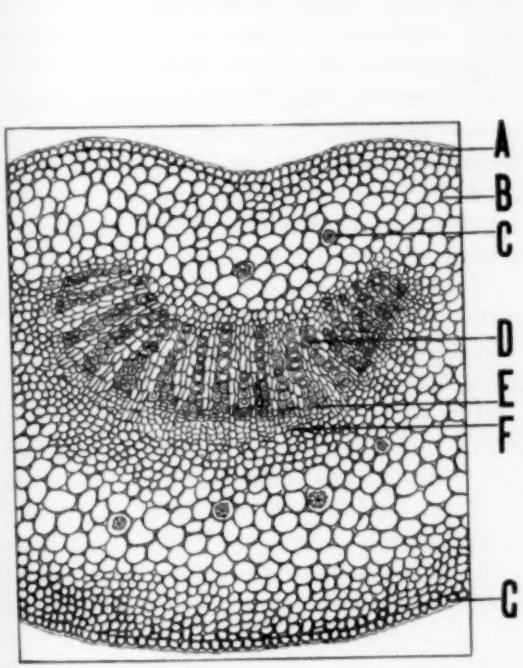

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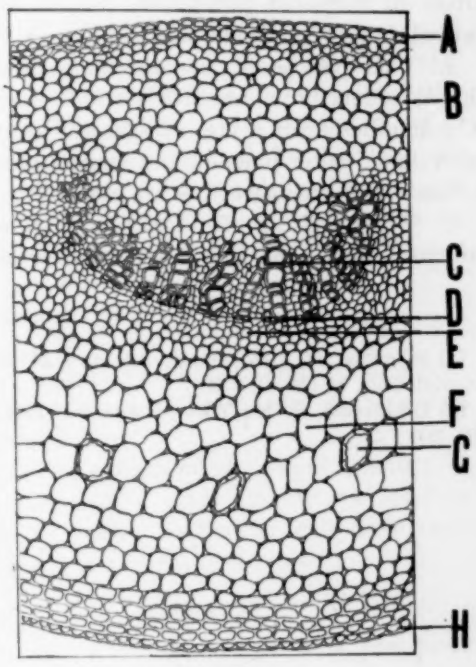

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PLATE IV

For description see opposite page.

ammoniacal solutions were then separated and diluted with 20 cc. of water. Both solutions retained a reddish-orange color. Cascara Sagrada when treated as above had more of a yellowish red color.

The barks of both *Rhamnus lanceolata* and *Rhamnus Frangula* L. gave a sublimate on slides, upon microsublimation. These sublimates were then dissolved in a small amount of alcohol and a few drops of ammonia T. S. added. In each case a weak orange-red color was produced.

R. Frangula L. produced color reactions which were deeper in intensity than those produced by a sample of Cascara Sagrada. The 3 samples were run simultaneously under identical conditions. It was noted the colors produced by *R. lanceolata* were the deepest, and the color produced by Cascara Sagrada, the weakest.

SUMMARY

1. This investigation deals with the anatomy of the stem, leaf and petiole of *Rhamnus lanceolata* Pursh and *Rhamnus Frangula* L. A brief discussion of the anthraquinone content of the 2 species is included.

2. The stem bark of *R. lanceolata* contains many groups of cortical and phloem fibers accompanied by crystal fibers bearing monoclinic crystals of calcium oxalate. Rosette aggregates are abundant throughout the bark. Sieve tubes have callous plates on both vertical and transverse walls. The wood is diffuse porous and consists entirely of bordered pored, scalariform and reticulate vessels, wood fibers and wood rays.

3. The petiole of *R. lanceolata* shows dorsal collenchyma beneath the epidermis. The bundles form a crescent-shaped group with dorsal outer phloem, and ventral inner xylem. Rosette aggregates are abundant.

4. The leaf of *R. lanceolata* shows stomata only on the lower epidermis. Directly beneath the upper epidermis is a double row of palisade parenchyma. Chloroplasts are nearly equally abundant in the palisade and spongy parenchyma. The structure of the midrib approximates that of the petiole.

5. The stem of *R. Frangula* L. has groups of pericyclic fibers and groups of phloem fibers accompanied by crystal fibers. Rosette aggregates are wanting. Phelloderm is often wanting. The wood is diffuse porous and consists of wood fibers, vessels, wood rays and a small amount of wood parenchyma.

6. The petiolar structure of *R. Frangula* L. is similar to that of *R. lanceolata* except that rosette aggregates are wanting in the former. *R. Frangula* L. has several schizogenous reservoirs in its dorsal cortex.

7. The leaf of *R. Frangula* L. has occasional unicellular, nonglandular hairs on its upper epidermis and stomata under the larger veins and midrib. Stomata occur only on the lower epidermis. There is a single layer of palisade. The structure of the midrib approximates that of the petiole.

8. A comparison of the barks of *R. Frangula* L., *R. lanceolata* and *R. Purshiana* shows that the barks of the 2 latter species are similar in many respects, while the bark of *R. Frangula* L. differs in having fibers in the pericycle and phloem only.

9. A study of the anthraquinone content of the barks of *R. Frangula* L. and *R. lanceolata* shows that on the basis of the U. S. P. tests their anthraquinone content appears to be higher than that of Cascara Sagrada U. S. P.

REFERENCES

(1) Solereder, H., "Systematic Anatomy of the Dicotyledons," 1 (1908), 218.

(2) Gathercoal, E. N., THIS JOURNAL, 4 (1915), 193, 360.

(3) Rusby, H. H., *Proc. A. Ph. A.*, 38 (1890), 203.

(4) Sayre, L. E., *Am. J. Pharm.*, 69 (1897), 126.

An Investigation of the Oil of *Cerbera odollam**

By Frank A. Steldt and K. K. Chen

During the course of our studies (1) on cerberin and cerberoside, which are the active principles of the seeds of *Cerbera odollam*, a quantity of oil was obtained. The material arrived in 2 batches. The physical constants and some of the constituents of the oil obtained from the first batch of the nuts were determined.

The extraction of the ground, dried nuts of *Cerbera odollam* with ethyl ether yielded 62% of a yellow-brown oil. The physical characteristics of this material were determined by the usual methods. The oil was extracted with ether. After evaporation *in vacuo*, it was found to solidify at 27–28° C. It began to melt at 29–30° C., and it was nondrying. It was optically inactive and had a refractive index of 1.4599 at 30° C. The specific gravity at 30° C. was 0.9031. The acid value was 73.75, the saponification value was 194.75, and the Hanus iodine number was 75.9. The Hehner values were: soluble acids as butyric, 0.71%; and insoluble and unsaponifiable, 90.0%. Duplicate determinations were made in each case. The figures, therefore, represent the averages.

The fatty acids present in the oil were separated as far as possible by fractional distillation of their ethyl esters under reduced pressure. Nine fractions were obtained at 14 mm. Hg, from 198–225° C., by use of a 27-cm. fractionating column packed with glass rings. The fractions were individually saponified, the acids liberated with sulfuric acid and then recrystallized from alcohol. It was not possible to obtain a single pure acid from each fraction in this way, but the preparation of the *p*-phenylphenacyl esters of the acids made the isolation and identification of palmitic acid from the first and second fractions possible. After several recrystallizations of the acids obtained from the remaining ethyl ester fractions by fractional distillation, as mentioned above, a point was reached at which two acids were present in each fraction. By titration with standard alkali, the neutralization equivalent of each fraction was determined. The composition of the fraction could then be elucidated by considering the neutralization equivalent and the melting point (2). It was found that stearic and palmitic acids are the chief saturated constituents of the oil.

An unsaturated liquid acid was found in the mother liquors from the recrystallization of the saturated acids. The neutralization equivalent showed it to be chiefly oleic acid.

The oil of the seeds of *Cerbera odollam* has been the subject of study by other investigators. De Vry (3) reported 44.8% of oil by expression and 57.8% by extraction with benzene. By extraction with petroleum ether, Plugge (4) found that the kernels contained 77% of oil. Ghanekar and Ayyar (5) reported that the nondrying oil, which was 43.1% of the seeds, consisted of the following glycerides: linolic acid 16.4%, oleic acid 4.2%, myristic acid 0.4%, palmitic acid 30%, stearic acid 9.9%, lignoceric acid 0.9%, and 0.8% unsaponifiable matter. The amount of oil in the kernels, as reported by Kafuku and Hata (6), was 64%, which is closer to our own figure. The fatty acids detected by these workers were myristic, palmitic and oleic acids.

CONCLUSIONS

1. The physical characteristics and constants of the oil of *Cerbera odollam* have been determined.

2. The glycerides of a small quantity of the lower fatty acids (as butyric) and of palmitic, stearic and oleic acids have been shown to be present in this oil.

REFERENCES

(1) Chen, K. K., and Steldt, F. A., *J. Pharmacol.*, 76 (1942), 167.

* Received Dec. 26, 1942, from the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

(2) Lewkowitsch, J., "Chemical Technology and Analysis of Oils, Fats, and Waxes," Fifth edition, Macmillan & Co., London, 1 (1913), 641.

(3) De Vry, J. E., *Sitzber. Akad. Wiss. Wien*, Abt. I, 49 (1864), 136; *Ibid.*, Abt. II, 49 (1864), 95.

(4) Plugge, P. C., *Arch. Pharm.*, 231 (1893), 10.

(5) Ghanekar, R. R. V., and Ayyar, P. R., *J. Indian Inst. Sci.*, 10A (1927), 20.

(6) Kafuku, K., and Hata, C., *J. Chem. Soc. Japan*, 55 (1934), 369; *Ibid.*, 57 (1936), 723.

The Rat as a Suitable Animal for the Study of Prolonged Medication*

By Robert C. Anderson, Francis G. Henderson and K. K. Chen†

One of the best means of estimating the toxicity of a drug by prolonged administration is to incorporate the drug in the food of animals. Such a procedure affords gradual medication and continuous absorption. It saves manual labor of individual dosing and avoids a peak-load effect of a pharmacologic reaction. The results often contribute information undemonstrable by single doses and throw much light on the clinical application of a new remedy, especially when administered repeatedly.

In the investigation of chemotherapeutic agents, Bieter, Larson, Levine and Cranston (1, 2), Litchfield, White and Marshall (3, 4, 5, 6) and Richardson (7) employed mice and fed them diets containing drugs. This proved equally satisfactory in our own laboratory. For the past 2 years, we have also used rats and found them to have certain good features which merit reporting here.

To carry out a series of experiments, rats are housed in individual cages, measuring 10 × 14 inches and 10 inches high. The food cup consists of a half-pint tin can, $2^3/_4$ inches in diameter, having a removable flanged top with a circular hole in center, $1^1/_2$ inches in diameter. The entire flange rises toward the edge at an angle of approximately 30° with the horizontal plane. The ration is composed of all the necessary ingredients and essential vitamins, adequate for the optimal growth of the animals. They are so well known that they need no enumeration. Drinking water is freely provided from a jar or an inverted bottle. As a rule, a group of 5 rats, 45 days old, is put on each concentration of the drug incorporated in food. The duration of study lasts 4 weeks or longer.

The obvious advantages of the rat in these experiments are as follows: First, the rat, unlike the mouse, is not in the habit of digging into food and wasting it, so that any decrease in the food cup usually represents the amount of food ingested by the animal. Accurate records of daily doses of the drug can thus be made. Secondly, control rats on the standard diet give rise to a smooth, progressive growth curve. Any leveling of the same in the treated groups may therefore be interpreted as evidence of untoward action of the drug. An example of such observations with sodium dilantin and sodium 5-phenyl-5-thienyl hydantoinate is shown in Fig. 1. It must be pointed out, however, that the material must be palatable to the animal and not bitter, otherwise the rat will refuse to eat and give false information on its growth.

Thirdly, the rat can stand repeated bleeding, amounting to 0.05–0.1 cc. each time, for blood chemistry or blood counts as routine criteria of investigation. In Table I, the results of 6 sulfonamides are summarized—sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, trichloro-acetyl sulfanilamide and N′-benzoyl sulfanilamide. Two to three days after the experiments had commenced, the blood concentrations of each compound in free form reached a maxi-

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† The authors are indebted to Dr. Paul N. Harris for his valuable assistance in conducting all autopsies and microscopic examinations.

TABLE I

Drug	Concentration in Food, %	Average Sustained Blood Concentration (Free Form), Mg./100 Cc.	General Effect
Sulfanilamide	0.5	8.0	Normal growth
	1.0	14.4	Growth slightly inhibited
	1.5	19.2	Growth impaired; 1 rat had slight hydrops
	2.0	46.2	Weight stationary; 1 rat showed dilated renal tubules
Sulfapyridine	0.5	10.1	Growth slightly retarded; 2 had calculi in bladder, ureters and kidney pelves
	1.0	17.7	Growth impaired; 1 died in 14 days; 3 out of 5 showed renal and ureteral lithiasis
	1.5	27.0	All lost weight; 1 died in 14 days; 4 out of 5 exhibited renal calculi
	2.0	35.7	Severe weight loss with 1 death in 13 days; all had bilateral hydronephrosis and hydroureter with calculi in renal pelves, ureters and bladder
Sulfathiazole	1.0	8.9	Slight impairment in growth; viscera normal
	2.0	35.0	No gain in weight; dilation of renal tubules in 1 case
	3.0	21.5	Loss of weight; 1 died in 9 days; all showed nephrolithiasis
	4.0	37.0	Marked weight losses and all died within 26 days; all had renal lithiasis
Sulfadiazine	1.0	23.5	Slight impairment of growth; 2 with crystals in medulla
	1.5	32.0	Definite growth retardation; all had crystals in medulla with lithiasis of tubules and hydronephrosis
	2.0	30.5	Slight loss in body weight; all showed crystals in medulla
	2.5	32.0	Marked weight loss; crystals in medulla with hydronephrosis
	3.0	32.0	Severe loss of weight; pathology similar to above
Trichloro-acetyl sulfanilamide	1.5	16.5	Severe weight loss; 1 died in 10 days; emaciation; 2 showed some calculi and hydronephrosis
	2.0	19.8	Definite weight loss; 1 out of 5 had some calculi
	2.5	23.5	Marked loss in weight; all survived but emaciated
	3.0	14.0	Emaciation in the entire group; 3 out of 5 died
	3.5	29.5	3 out of 5 died in 10 days; emaciation in all 5
N'-Benzoyl-sulfanilamide	1.0	17.6	Normal growth
	1.5	24.5	Normal growth; no pathologic changes
	2.0	23.1	Growth retarded; 3 out of 5 showed slight hydrops but no calculi
	2.5	28.1	Growth impaired; viscera of 3 normal; 2 had obstructions of renal collecting tubules but no calculi in pelves or ureters

TABLE II

Drug	Concentration in Food, %	General Effect
Isomannide	5.0	Normal growth; no pathologic changes
	10.0	Normal growth; no pathologic changes
Isomannide dinitrate	0.1	Normal growth; no pathologic changes
	0.5	4 appeared normal; 1 died on 10th day—fatty metamorphosis of liver and kidney
	1.0	Growth retarded; 2 died after 10 and 15 days; pathologic picture similar to one that died with 0.5% of the drug
	1.5	3 out of 5 died in 20 days; pathology similar to those on 1% of the drug; urine slightly tinged with blood; 2 survivors emaciated but without tissue damage
Sodium dilantin	0.5	Retardation of growth
	1.0	Growth definitely retarded
Sodium 5-phenyl-5-thienyl hydantoinate	0.5	Normal growth
	1.0	Retardation of growth
Senecionine	0.001	Normal growth
	0.005	Growth retarded; 3 out of 5 died within 24 days; liver necrosis
	0.010	Loss of weight; 2 out of 5 died in 9 days; pathology similar to above group
	0.050	Deaths in all cases with central necrosis of the liver
Dicumarol	0.005	2 out of 5 died and showed central necrosis of the liver
	0.010	4 out of 5 died; 1 had central necrosis of the liver
	0.050	All 5 died; 4 had central necrosis of the liver
	0.100	All 5 died; 2 had liver necrosis centrally
	0.500	All 5 died; 2 showed central necrosis of the liver
	1.000	All 5 died; 2 had liver necrosis in central areas

mum and remained constant throughout the entire period of study until the kidneys became severely damaged. With a few exceptions, as shown in Table I, the higher the concentration of the drug in food, the larger the amount of the drug in circulation.

amide and N′-benzoyl sulfanilamide but none with sulfanilamide. It was further observed, as illustrated in Fig. 2, that sulfapyridine was more apt to produce renal lithiasis than sulfathiazole. No abnormal effects resulted with isomannide as high as

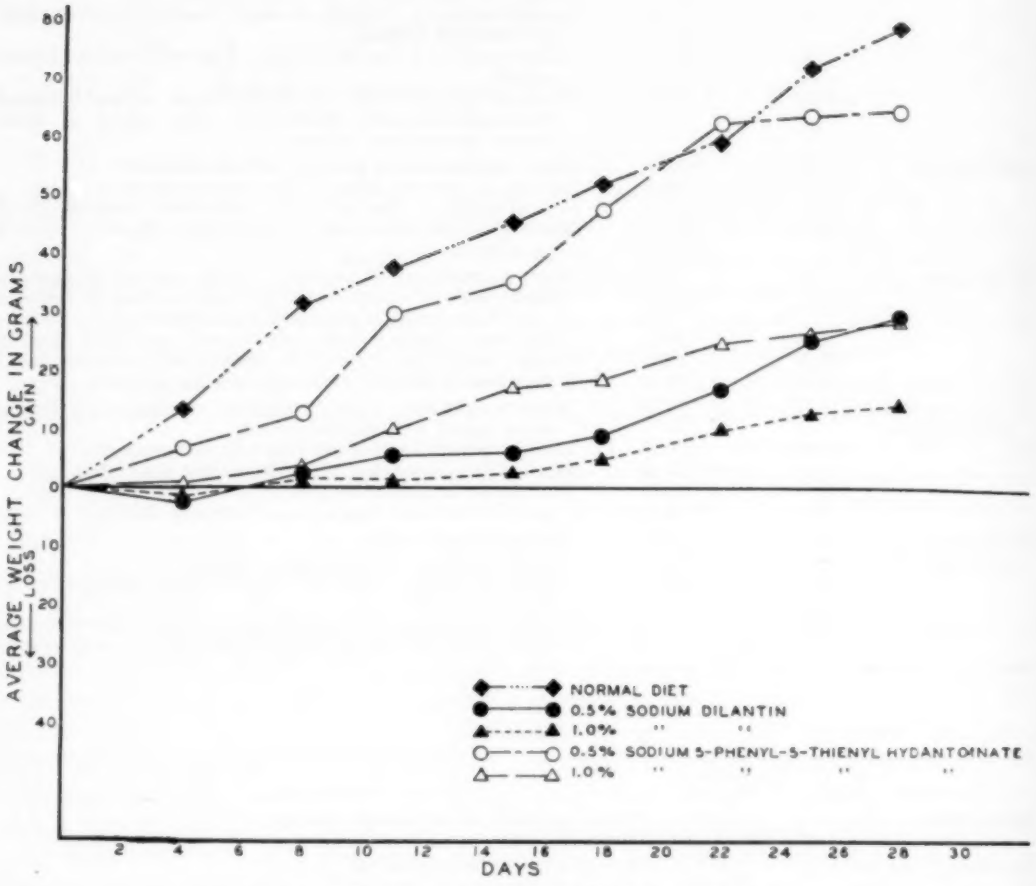

Fig. 1.—Influence of Drugs on the Rat's Growth Curve.

Fourthly and lastly, pathologic lesions can be progressively induced if the drug has any deleterious effects on the organs and tissues. Of the 6 sulfonamides listed in Table I, renal damages occurred with sulfapyridine, sulfathiazole and sulfadiazine, very rarely with trichloro-acetyl sulfanil-

10% in food, but slight fatty metamorphosis of the liver and kidneys appeared with isomannide dinitrate in concentrations of 0.5–1.5%. Both isomannide and isomannide dinitrate were previously investigated by Krantz and his associates (8, 9, 10). Of the numerous *Senecio* alkaloids studied

Fig. 2.—Renal Damage Caused by Sulfonamides

A. Rat, female, ingested 1% sulfapyridine continuously, and died on the 16th day. Note the crops of crystals at the kidney pelvis.

B. Rat, female, ingested 2% sulfathiazole continuously for 28 days. It was then sacrificed. The kidney pelvis is practically normal.

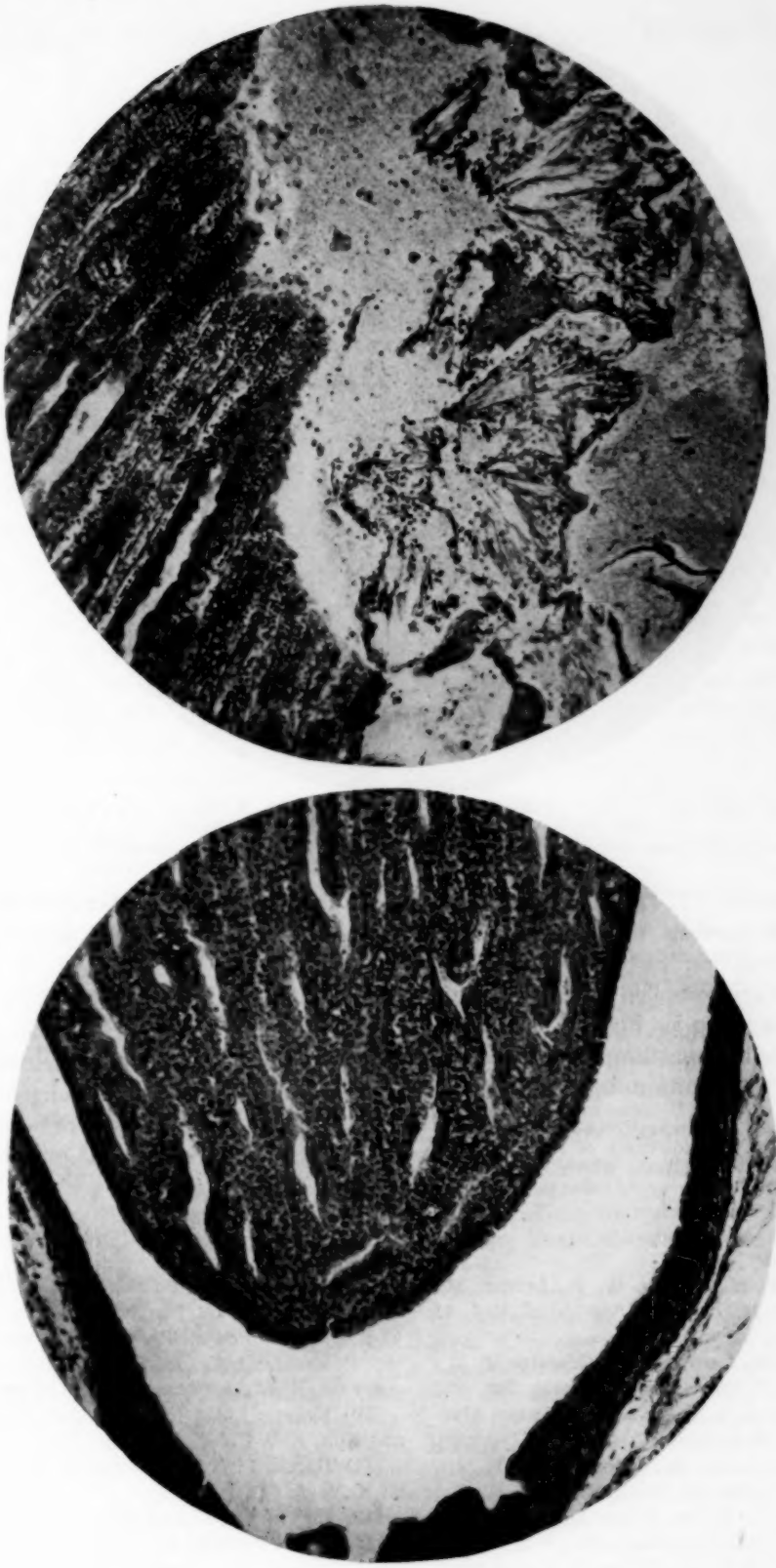

FIG. 2. (*See opposite page.*)

Fig. 3.—Liver Damage Produced by Senecionine
Rat, female, was fed a diet containing 0.1% senecionine. It died on the 6th day. Note the central necrosis of the liver.

in this laboratory (11, 12), senecionine produced central necrosis of the liver in as low a concentration as 0.005% in food. The protocols are compiled in Table II, and an example is given in Fig. 3. Similarly, Dicumarol,[1] a hypoprothrombinemic agent (13), caused, in addition to hemorrhages, central necrosis of the liver in 0.005–0.01% in food. The group results are enumerated in Table II.

[1] Dicumarol, 3,3′-methylenebis(4-hydroxycoumarin), is the collective trade-mark of the Wisconsin Alumni Research Foundation, which controls the use thereof.

SUMMARY

The rat has been proposed as the testing animal for the study of prolonged medication. Certain advantages have been pointed out, and examples with 12 products have been given.

REFERENCES

(1) Bieter, R. N., Larson, W. P., Levine, M., and Cranston, E. M., *Proc. Soc. Exptl. Biol. Med.*, 41 (1939), 202.

(2) Bieter, R. N., Larson, W. P., Cranston, E. M., and Levine, M., *J. Pharmacol.*, 68 (1940), 252.

(3) Litchfield, J. T., Jr., White, H. J., and Marshall, E. K., Jr., *Ibid.*, 67 (1939), 437.

(4) Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J., *Ibid.*, 69 (1940), 89.

(5) Litchfield, J. T., Jr., White, H. J., and Marshall, E. K., Jr., *Ibid.*, 69 (1940), 166.

(6) Litchfield, J. T., Jr., White, H. J., and Marshall, E. K., Jr., *Ibid.*, 72 (1941), 291.

(7) Richardson, A. P., *Ibid.*, 67 (1939), 429.

(8) Krantz, J. C., Jr., Evans, W. E., and Carr, C. J., *Quart. J. Pharm. Pharmacol.*, 8 (1935), 213.

(9) Krantz, J. C., Jr., Carr, C. J., Forman, S., and Ellis, F. W., *J. Pharmacol.*, 67 (1939), 187.

(10) Krantz, J. C., Jr., Carr, C. J., Forman, S. and Ellis, F. W., *Ibid.*, 67 (1939), 191.

(11) Harris, P. N., Anderson, R. C., and Chen, K. K., *Ibid.*, 75 (1942), 69.

(12) Harris, P. N., Anderson, R. C., and Chen, K. K., *Ibid.*, 75 (1942), 83.

(13) Rose, C. L., Harris, P. N., and Chen, K. K., *Proc. Soc. Exptl. Biol. Med.*, 50 (1942), 228.

Behavior of Solutions of Morphine Salts When Stored in Tin Containers*

By W. G. Christiansen and A. E. Jurist

The behavior of morphine solutions in tin containers is of much importance because units containing single doses with needle attached represent an emergency means of administration. Two obvious requisites for such a product are: stability of the morphine content and absence of corrosion of the tin by the solution. Jermstad and Gaule (1) included morphine hydrochloride in a study of tin corrosion by drug solutions and concluded that there was no corrosion. Their tests were done on the solutions after filtration and the kind of tests employed did not show the presence of any dissolved tin. For our solutions, morphine sulfate was chosen and we soon found that, in spite of an initial condition of brilliant clarity and thoroughly clean tin surfaces, a haze or milkiness existed before long (the time within which this happened being much too short to consider the product acceptable). Such solutions when clarified and assayed for morphine showed no loss of potency indicating that the drug had not been affected, but by chemical test it was shown that the material responsible for the haze or milkiness contained tin, presumably a tin oxide or basic tin salt. This of necessity meant that the solution had corroded the tin surface. To correct this, the morphine sulfate solution might be adjusted so as to be less corrosive toward tin or a salt other than the sulfate might be employed. Both devices were explored.

Buffering morphine sulfate solutions to lower acidities merely slows down the corrosion (does not prevent it) and at the same time introduces another kind of difficulty, for the solutions discolor, suggesting deterioration of the alkaloid. This observation regarding discoloration is in line with one by Berry (2) to the effect that alkaline morphine solutions discolor more readily than acid ones. While this did not turn out to offer a promising solution to the problem, good results were had by employing the organic salts instead of the sulfate.

The organic acid salts of morphine investigated include the acetate, citrate, lactate and tartrate, the latter giving especially good results. With none of these was there any serious loss of clarity, although the lactate did show slight haze after long storage. The acetate, citrate and lactate were not completely satisfactory because with them slight discoloration occurred. That, however, did not happen with the tartrate. It remained colorless as well as clear. In view of the fact that these conclusions are based on intermittent observations of solutions kept in tin containers at semitropical temperatures for $1^1/_2$ to 2 yrs., it may be assumed that these solutions will be indefinitely stable.

EXPERIMENTAL

The morphine salt solutions were filled into tin containers which were then sealed by welding. All solutions contained 0.4% phenol as a preservative and $^1/_2$ grain of the morphine salt per $1^1/_2$ cc. of solution. In all cases except morphine lactate, the pure morphine salts were used. Morphine lactate was prepared from morphine alkaloid and the proper amount of lactic acid, U. S. P. Solutions were stored both in the laboratory at room temperature and in a constant temperature oven at 105° F. At various intervals, some of the containers were opened and the contents examined, carrying out morphine assays wherever the circumstances warranted.

Buffered morphine sulfate solutions were prepared by adding sufficient amounts of sodium phosphate, U. S. P. to raise the *p*H of the solutions to 6.0. Any adjustment of the *p*H above this point results in precipitation of morphine alkaloid.

Table I shows the physical appearance of the solutions after the indicated storage periods, and Table II shows the results of the morphine assays which are expressed in percentage of morphine salt recovered as compared to the stated concentration of $^1/_2$ grain in $1^1/_2$ cc. of solution.

In another experiment, a morphine sulfate solution in its tin container was heat-sterilized by auto-

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* From the Development Division of the Chemical and Pharmaceutical Laboratories, E. R. Squibb and Sons, Brooklyn, N. Y.

Presented to the Scientific Section of the A. PH. A., Denver meeting, 1942.

TABLE I.—APPEARANCE OF MORPHINE SOLUTIONS AFTER STORAGE

Nature of Morphine Salt	Length of Storage Period	Storage at Room Temperature	Storage at 105° F.
Sulfate	6 weeks	Colorless, hazy	Colorless, hazy
Sulfate, buffered to *p*H 6.0	1 month	Yellow tinted, white specks	Colorless, white specks
Sulfate, buffered to *p*H 6.0	2 months	Yellow, hazy	Hazy, some white specks
Acetate	23 months	Discolored, clear	Discolored, faintly hazy
Citrate	20 months	Faint green tint, clear	Faint green tint, clear
Lactate	20 months	Yellow tint, hazy	Yellow tint, hazy
Tartrate	20 months	Colorless, clear	Colorless, clear

TABLE II.—ASSAYS ON MORPHINE SOLUTIONS AFTER STORAGE

Nature of Morphine Salt	Length of Storage Period	Storage Conditions	Recovery of Morphine Salt, %
Sulfate	12 months	Room temp.	98.27
Tartrate	11 months	Room temp.	96.64
Tartrate	11 months	105° F.	95.83

claving for ½ hr. at 15 lbs. steam pressure. It then contained some insoluble matter on which a positive test for tin was obtained, but the morphine assay came out 99.64% of theory.

It will be noted from the above data that the results obtained with morphine tartrate were satisfactorily both from the standpoint of the physical stability of the solution and the stability of the morphine content.

REFERENCES

(1) Jermstad and Gaule, *Schweiz. Apoth.-Ztg.*, 57 (1919), 89, 109.

(2) Berry, *Public Pharmacist* (1941), through *Pharm. J.*, 146 (1941), 231.

Polarographic Determination of Lead in the Contents of Collapsible Tubes*

By David F. Menard†,‡

The use of tin in collapsible tubes has been drastically reduced since early in 1942. Analytical methods for the determination of lead in the contents of such tubes are of considerable importance because that metal is the only substitute for tin in general use at present.

Restrictions on the Use of Tin.—The initial restriction of the War Production Board on the use of tin for collapsible tubes was made in January, 1942 (1). Since then further restrictions have appeared at approximately quarterly intervals. At the time of writing this paper, the restrictions are as follows (2):

1. Proscription of a few very particular pharmaceuticals such as ophthalmic and sulfonamide preparations, Class I products, which may be packaged in tubes of pure tin.

2. Reduction of the tin content of collapsible tubes for a large group of preparations by (*a*) limiting all medicinals and pharmaceuticals, Class II products other than Class I, to a maximum of 7.5% tin; (*b*) limiting dental cleaning preparations, Class III products, to a maximum of 3.0% tin.

3. Proscription of tin in any form for packaging the remainder. Examples of these "non-essential" products include cosmetics, shaving preparations, and liquid cements.

Substitute Metals for Tin.—In the manufacture of tubes containing 1.5% to 10% tin, the customary practice has been to use lead slugs having a layer of tin on all surfaces. These slugs are then extruded into tubes in the usual manner. The resulting "tin-

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coated lead" tubes have a coating of tin on both the interior and exterior surfaces and have the same shiny appearance as a pure tin tube. But these coatings are discontinuous almost without exception, although the discontinuity is difficult to discern with the naked eye on tubes containing 10% tin.

One alternative has been to make tin-lead alloys containing the maximum permissible quantity of tin. This practice has not been widely adopted, however, because the alloy tubes have seldom shown any significant advantages over ordinary lead tubes in appearance or packaging qualities.

Internally Waxed Tubes.—For many products, the tin coatings in 7.5% tin-coated lead tubes were found to be of sufficient continuity to prevent serious absorption of lead by the contents. Restrictions or proscription of tin and the possibility of further restrictions have led to the investigation of the use of other methods to prevent lead adsorption.

At present, the most widely used method is to flush or spray a wax coating on the interior surface of lead or tin-coated lead tubes. This method of protection has been satisfactory for many products, especially preparations of an aqueous nature such as dentifrices or shaving cream.

EXPERIMENTAL

General Analytical Considerations.—The analytical procedure most generally used for determining small quantities of lead is the colorimetric dithizone procedure of the Association of Official Agricultural Chemists (3). This method, which is well described in the literature (4, 5), is based on the selective extraction of lead from aqueous ammonium citrate-cyanide solutions by a chloroformic solution of dithizone ($C_6H_5NH—NH—CS—N:NC_6H_5$). The dithizone-lead complex thus formed is then decomposed by dilute acid and the lead determined by a colorimetric procedure with dithizone.

The metallic ions which interfere with the colorimetric method are zinc (6, 7), stannous tin (8, 9), bismuth and thallium. Bismuth and thallium, however, are found so rarely that they may be ignored in general. It seemed logical to the author that lead determinations could be made with reasonable accuracy on a polarograph, thus eliminating colorimetric comparisons for determining the final results as well as interference from zinc and stannous tin. In the polarographic method the tin is held in the stannic form and zinc causes no interference (10). The polarographic method is not new, having been discovered about 20 years ago by J. Heyrovsky (11). It is applicable to all substances which are electroreducible in the range of 0–2 v. If fine globules of mercury are dropped into specific solutions, each metallic ion produces a current discharge at definite voltages; the quantity of current thus produced, measurable on a galvanometer, is proportional to the concentration of the metallic ion. The theoretical and practical aspects of polarography have been well described by the publications of Kolthoff and Lingane (10).

The method set forth in this paper is a combination of the dithizone procedure (3) and polarography. The isolation of lead is accomplished by the A. O. A. C. method (3) to the point where that element has been completely extracted with dithizone for the first time. Then the dithizone-lead complex is decomposed with dilute acid and the lead in the acid extract is determined on a polarograph.

Apparatus and Reagents.—The apparatus required for the isolation of lead is the same as used in the A. O. A. C. method (3). The "Elecdropode," a dropping mercury electrode instrument made by the Fisher Scientific Co., is suitable for the polarographic work. The reagents require no special purification other than as suggested in the A. O. A. C. method (3). Lead-free water is prepared either by redistillation in a Pyrex apparatus or by the method of T. D. Gray (12).

Tubes Used to Package the Products Tested.—The collapsible tubes utilized to package the several varieties of products were prepared early in 1941 in anticipation of restrictions on the use of tin. The present allowable quantities of tin (2) were overestimated, as shown from the description of their composition in Table I:

TABLE I.

Pure tin (0.2–0.4% Cu)
Tin-coated lead (10% Sn)
Tin-lead alloy A (12% Sn, 3% Sb, 1% Cu, 84% Pb)
Tin-lead alloy B (6% Sn, 94% Pb) for nitrocellulose cements only
Silver-lead alloy C (1% Ag, 3% Sb, 96% Pb) for mascara only
Lead (97% Pb, 3% Sb)

All these tubes were made both with and without internal wax coatings, except in the instances of tin, alloy B and alloy C tubes. The wax used was a commercial grade of a brown amorphous petrolatum wax, m. p. 155° F. Some of the products were also packaged in glass jars for control samples. The products packaged in these tubes were tooth paste, brushless shaving cream, lather shaving cream, vaginal jelly, scalp cream, mascara, nitrocellulose cement, and several varieties of ointments. In all cases the tubes were filled and closed by commercial tube fillers.

Incubation and Sampling.—Except as indicated elsewhere in this paper, the tubes were all incubated for three months at 100° F., assumed to be equiva-

lent to a year's storage on store shelves in temperate climates (13). Then the contents of the tubes were extruded into ashing casseroles by squeezing the

Fig. 1.

1, Blank on solutions used. *2*, Blank on jelly containing 24 micrograms lead. *3*, Same jelly plus 100 micrograms lead. *4*, Same jelly plus 200 micrograms lead. *5*, Same jelly plus 300 micrograms lead.

tubes flat until they were nearly empty. The samples thus taken generally weighed 15–35 Gm.

Ashing and Solution of the Ash.—Some of the products required special methods of charring. Solvents were removed from nitrocellulose cement by evaporation on a steam bath. The nitrocellulose was then decomposed with 1 cc. of nitric acid per Gm. of original sample on a steam bath. Most other products were dehydrated several hours in a 110° C. oven, followed by charring on a hot plate or over a small flame. The lather shaving creams foamed so vigorously at 110° C. that they were partially dehydrated at 80° to 100° C. followed by charring directly over a small flame. Considerable caution was necessary to prevent overheating and flashing of petrolatum-base ointments. Brushless shaving cream and analgesic balm required about 3 hr. evaporation at 150° C. to prevent spattering. After ashing several hours at not over 500° C., the A. O. A. C. method (3) for dissolving the sample was followed. The ash was treated with 2 to 3 cc. of "ash aid," followed by ashing for 30 min. at 500°. This treatment was repeated using 2 to 3 cc. of nitric acid. The residual material was usually soluble in hydrochloric acid and contained no silica, so that dehydration with perchloric acid was unnecessary. The hydrochloric acid solution was filtered through a sintered glass funnel, and the casserole leaching procedure of the A. O. A. C. was continued with 10 cc. each of hydrochloric acid, hydrochloric acid-citric acid, and 40% ammonium acetate, filtering each through the same sintered glass funnel. The residues in the casseroles were then leached with hot 50% sodium hydroxide, hydrochloric acid, and water in succession, adding these washings directly to the filtrate.

Extraction with Dithizone.—This step was also carried out according to the A. O. A. C. method. If the second extraction with 20 cc. of dithizone solution of 0.1 Gm. dithizone per 1000 cc. of chloroform remained bright red, indicating a large quantity of lead present, the next extraction was made with 20 cc. of dithizone solution, of 1.0 Gm. dithizone per 1000 cc. of chloroform.

Removal of Lead from the Dithizone Complex.—In many instances the lead was incompletely extracted from the dithizone complex with one 110-cc. portion of 1% nitric acid (14). Extraction with dilute hydrochloric acid was also found to be incomplete. Where three or four 40-cc. portions of 1% nitric acid were used instead of one 110-cc. portion, all the lead was brought into the aqueous phase. This finding agrees with recent results of Schultz and Goldberg (15).

The combined acid extracts were evaporated *just to dryness* on a hot plate over a low flame. Where too much heat was used, the residue was difficult to dissolve. If the residue was not dry, the results on the polarograph were poor. Direct determination of the lead in the acid extracts gave very erratic results on the polarograph. Where the lead content of a sample was high as indicated in its dithizone extraction, the acid extract was diluted to 200 cc. in a Pyrex volumetric flask and an aliquot, usually 50 cc., was evaporated to dryness.

Polarographic Determination of Lead.—The residue in a 100–150-cc. beaker was dissolved in 4.0 cc. of 0.13 *M* potassium chloride, and poured directly into

the cell of the polarograph. The beaker was then rinsed in order with 4.0 cc. of 0.13 *M* potassium chloride, 3.0 cc. of 0.13 *M* potassium chloride plus 1.0 cc. of water, and 3.0 cc. of 0.13 *M* potassium chloride. These solutions were all added to the cell. The addition of 2 drops of 1% nitric acid to the first or second potassium chloride rinse solution assisted in dissolving difficult samples. Then 2.0 cc. of cadmium chloride "pilot ion" solution (90 micrograms Cd per cc.) was added from a measuring pipette, followed by 4–6 drops of freshly prepared 4% gelatin solution. The *p*H was adjusted to 3 (*p*H test paper) with 1% nitric acid and nitrogen was bubbled through the cell for at least 10 min. During this time the cell temperature was adjusted to 25° C., plus or minus 0.5°.

TABLE II.

Micrograms Lead	
Added	Found
0	2
100	102
200	204
400	403

The usual polarographic determination of both lead and cadmium was then made as described by Feicht, Schrenck and Brown (16). The minima-maxima range of the lead curve was generally found between −0.420 v. and −0.550 v., and the cadmium between −0.630 v. and −0.720 v. Two readings were taken at 0.010-v. intervals on both sides of these voltages to locate the minima and maxima points more definitely. The cadmium maximum was seldom well defined.

RESULTS

Calculation of Results.—The Pb/Cd ratio was determined from the following galvanometer readings:

$$\frac{(\text{amps. at } -0.550 \text{ v.}) - (\text{amps. at } -0.420 \text{ v.})}{(\text{amps. at } -0.720 \text{ v.}) - (\text{amps. at } -0.630 \text{ v.})}$$

The average Pb/Cd ratio was taken from determinations at full, half and one-fifth sensitivities on the polarograph. Where the lead content was unusually large, causing the galvanometer to swing far off the scale, a reading at one-tenth sensitivity was substituted for full sensitivity. Figure 1 illustrates typical current-voltage relationships.

Similar determinations were made on lather shaving cream containing known added quantities of lead. The results are shown in Table II.

From results on other products containing known added quantities of lead and from reports of other workers (10, 16), this polaro-

TABLE III.—TOTAL LEAD (PARTS PER MILLION) IN TUBES (SEE TABLE I)

Product	Tin C.	Tin-coated Lead	Tin-coated Lead (waxed)	Alloy A	Alloy A (waxed)	Lead	Lead (waxed)
Tooth paste A	3	4	2	4	4	10	7
Tooth paste B	3	..	..	...	..	14	..
Lather shaving cream A	4	1	5	59	23	54	39
Lather shaving cream B	1	..	..	...	..	34	..
Brushless shaving cream A	3	..	4	...	..	..	..
Brushless shaving cream B	1	..	..	...	..	12	..
Vaginal jelly A	10	45	4	30	7	36	9
Vaginal jelly B	14	57	4	71	14	34	..
Scalp cream A	8	20	13	56	11	79	12
Scalp cream B	..	..	..	...	..	25	4
Nitrocellulose cement A	4	32	..	756[a]	..	..	..
Nitrocellulose cement B	..	10	4	412[a]	..	..	..
Burn ointment A	0.2	21	4	18	13	45	35
Burn ointment B	..	3[c]	2[c]	...	..	..	..
U. S. P. ammoniated mercury ointment	..	..	3[c]	...	..	..	1[c]
U. S. P. blue ointment	..	..	7[c]	...	..	..	3[c]
U. S. P. boric acid ointment	..	..	1[c]	...	..	..	3[c]
U. S. P. white petrolatum	..	..	2[c]	...	..	..	3[c]
N. F. Lassar's paste with salicylic acid	..	..	2[c]	...	..	..	3[c]
N. F. Ichthammol	..	..	10[c]	...	..	..	12[c]
Ichthyol	..	..	4[c]	...	..	..	6[c]
Analgesic balm	..	..	5[c]	...	..	..	74[c]
Black mascara	0	..	..	132[b]	..	14	3
Blue mascara	8	..	..	50[b]	..	51	17
Brown mascara	10	..	..	105[b]	..	39	19.6

[a] The tubes for nitrocellulose cement were alloy B instead of alloy A.
[b] The tubes for mascara were alloy C instead of alloy A.
[c] The tin-coated lead tubes for these ointments contained 7.5% tin. The wax used for lining them and the corresponding ead tubes was a mixture of 75% "ceresin" (white paraffin m. p. 135° F.) and 25% white petrolatum. The tubes containing the ointments were stored for 1 year at moderate room temperature, instead of 3 months at 100° F.

graphic method is regarded as accurate to plus or minus 5%. It should be satisfactory for routine commercial analyses of the contents of collapsible tubes.

Actual recoveries of lead obtained in the analysis of 25 products are given in Table III.

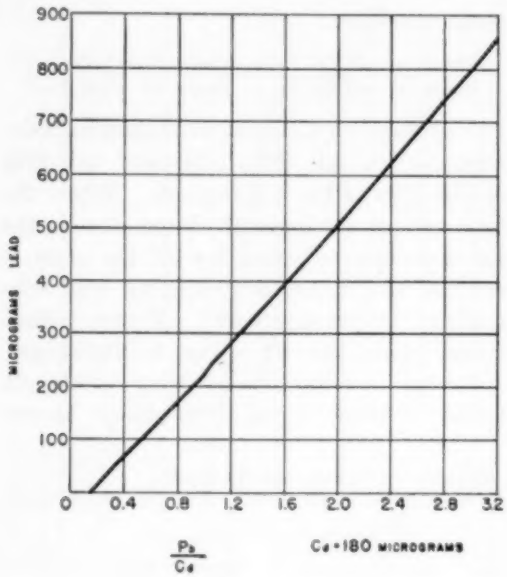

Fig. 2.

Control Samples.—Control samples of some products were taken as soon as received. The lead contents are given in Table IV.

Discussion of Results.—In some products of an acidic nature, such as vaginal jellies, the lead absorption was higher in the tin-coated lead tubes than in plain lead tubes. This difference might be explained by the assumption that, under these conditions, a tin-lead electrolytic couple was set up which was more active than lead alone.

Several important manufacturing variables which influence the absorption of lead have not been taken into consideration in this paper. Two of these variables are the thickness of the wax coating and the nature of the wax used. Since 1941, when these tubes were prepared, marked improvement has been made in both the composition of the wax and film thickness. For example, one ointment investigated was packaged in 7.5% tin-coated lead tubes having (1) no internal coating, (2) a low melting ordinary petroleum wax, and (3) a special tube wax, m. p. 165° F.; the lead absorbed by this inactive ointment was 5 p. p. m. in the bare tube, 3 p. p. m. in the tube coated with low m. p. wax, and 1 p. p. m. in the tube coated with the special wax. The low m. p. wax dissolved completely in the ointment but the special wax was insoluble

TABLE IV.—LEAD (PARTS PER MILLION)

Product	Container	
	Glass	Tin Tube
Tooth paste A	...	2
Tooth paste B	3	...
Lather shaving cream B	1	...
Brushless shaving cream A	...	1
Brushless shaving cream B	1	...
Vaginal jelly A	0.05	...
Scalp cream A	...	7
Scalp cream B	4	...
Nitrocellulose cement A	...	2
Nitrocellulose cement B	4	...
Burn ointment A	...	0.2
Burn ointment B	0.7	...
Blue mascara	6	...

REFERENCES

(1) War Production Board Conservation Order M-43, Jan. 15, 1942.

(2) *Ibid.*, M-115, sec. 110, 80f., Jan. 13, 1943.

(3) "Official and Tentative Methods of Analysis", Assoc. Official Agr. Chem., 23 (1940) 394.

(4) Clifford, P. A., and Wichmann, H. J., *J. Assoc. Official Agr. Chem.*, 19 (1936), 130.

(5) Bambach, K., *Ind. Eng. Chem., Anal. Ed.*, 11 (1939), 400.

(6) Clifford, P. A., *J. Assoc. Official Agr. Chem.*, 20 (1937), 215.

(7) Clifford, P. A., *Ibid.*, 23 (1940), 309.

(8) Laug, E. P., *Ibid.*, 21 (1938), 483.

(9) Wichmann, H. J., *Ibid.*, 23 (1940), 295.

(10) Kolthoff, I. M., and Lingane, J. J., "Polarography," Interscience Publishers, New York, 1941, pp. 267, 329.

(11) Heyrovsky, J., *Phil. Mag.*, 45 (1923), 303.

(12) Gray, T. D., *Ind. Eng. Chem., Anal. Ed.*, 14 (1942), 111.

(13) Maier, C. E., private communication.

(14) Clifford, P. A., *J. Assoc. Official Agr. Chem.*, 20 (1937), 622.

(15) Schultz, J. and Goldberg, M. A., *Ind. Eng. Chem., Anal. Ed.*, 15 (1943), 158.

(16) Feicht, F. L., Schrenck, H. H., and Brown, C. E., *U. S. Bur. Mines Rept. Investigations* No. 3639, May, 1942.

An Efficient and Inexpensive Method for the Biological Assay of Tincture of Aconite*

By James O. Hoppe and C. E. F. Mollett†

It has been pointed out (1) that the official guinea-pig method of assay for aconite, while being satisfactory from a theoretical standpoint, has a disadvantage from a practical viewpoint in that it is more expensive to conduct than the importance of small lots of the drug really warrants.

Rowe (1) has developed a technique using white mice instead of guinea pigs. This method is similar to the official procedure except that the mice are injected intraperitoneally instead of subcutaneously.

Since white mice cost one-tenth as much as guinea pigs, and in addition, are quite prolific and easy to maintain it was deemed advisable to investigate further the possibility of using the white mouse as a test animal. A large colony of mice can be readily developed and the cost of maintenance averages about 2 cents per mouse per mo.

In the work which has been published on the possibility of utilizing the mouse as a test animal, no specific attempt to assay aconite preparations on the basis of comparison with reference aconitine has appeared. Therefore, in this work, the lethal effect of reference aconitine was determined and used as a basis for an assay comparing this result with several lots of tincture of aconite.

In order to investigate the reliability of the white mouse, statistical methods of interpretation of experimental data would appear to give the most critical appraisal of the mouse method.

EXPERIMENTAL

Several official tinctures recently standardized and adjusted according to the official method were obtained from reliable pharmaceutical concerns.

To determine the accuracy of this method, each tincture was assayed several times using the LD_{50} for reference aconitine as a basis for standardization. The end point selected in this work was based on an LD_{50} that is, the doses of the reference aconitine and the preparation being assayed which killed 50% of the animals injected, at the end of a 24-hr. observation period. This does not leave anything to the chance of error due to individual interpretation, the animals are either alive or they are dead at the end of the 24-hr. observation period.

Mice, all from the same strain and sex at random, were selected from the colony of mice kept under identical conditions and fed on an adequate and consistent diet. The weight of the mice should not be less than 17.5 Gm. and not more than 22.5 Gm. for optimum results.

A dilution of the tincture to be assayed was prepared with distilled water in such a manner that the size of the dose ranged from 0.5 cc. to 0.75 cc. Injection was made intraperitoneally into the right lower quadrant of the belly of the mouse, holding the animal head down.

The solution of reference aconitine was prepared as is directed in the official method of assay in the Seventh Edition of the National Formulary. This was likewise diluted in the manner described above, to give the same volume of dose.

A graduated series of doses of the reference standard and the diluted tinctures were injected intraperitoneally and the LD_{50} determined, after a 24-hr. period. The strength of the tincture being assayed is then determined by direct proportion between the concentration of the tincture and the amount of reference aconitine that resulted in an LD_{50} in both cases. The official requirement is that 1 cc. of tincture be equivalent in action to 0.15 mg. of reference aconitine and this is taken to represent 100% of the required potency.

In Table I, the results of the assay of 4 tinctures are given. The widest variation is 16% which appears well within the present requirements of the N. F. VII which allows a variation of ±20% by which 2 official tinctures can vary in strength by 40% in terms of the official standard.

TABLE I.—PERCENTAGE STRENGTH OF TINCTURES ASSAYED BY MEANS OF THE MOUSE METHOD

	Preparation			
	Tincture A	Tincture B	Tincture C	Tincture D
Percentage strength	114.0	118.0	86.6	67.0
	98.0	120.0	84.0	70.6
	114.0	113.0	83.3	
Av., %	108.6	117.0	84.6	68.8

Statistical interpretation of data as shown in Table II, has shown that mice are reasonably accu-

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† Received March 1, 1943, from the School of Pharmacy, Montana State University, Missoula, Mont.

rate in the biological assay of tincture of aconite. The standard error in a series of assays on the same tincture, or upon reference aconitine was found to be approximately 1%.

under the same conditions have died had it received any higher dose and (2) an animal which survives a given dose would also have survived had it received a smaller dose. In using this double integration

TABLE II.—STATISTICAL INTERPRETATION OF DATA

Formula	Reference Aconitine (Mg./Gm. Body Weight)	Preparation Bioassayed: Tincture of Aconite (Cc./Gm. Body Weight)			
	Aconitine	A	B	C	D
(3) $M = \frac{\Sigma X}{n}$	0.0003962	0.0023062	0.0022252	0.00313	0.00385
(4) $A.D. = \frac{\Sigma d}{n}$	6.6×10^{-4}	2.91×10^{-3}	2.58×10^{-3}	4.3×10^{-3}	5.0×10^{-3}
(5) $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$	8.7×10^{-4}	3.104×10^{-3}	2.97×10^{-3}	4.7×10^{-3}	5.0×10^{-3}
(6) $P.E. = \frac{2\sigma}{3}$	5.8×10^{-4} (1.4%)	2.069×10^{-3} (0.89%)	1.98×10^{-3} (0.89%)	3.1×10^{-3} (0.99%)	3.3×10^{-3} (0.85%)
(7) $S.E. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$	3.3×10^{-4} (0.8%)	2.19×10^{-3} (0.94%)	2.01×10^{-3} (0.903%)	3.0×10^{-3} (0.95%)	5.0×10^{-3} (1.3%)

The method described by Wright (2) for the statistical interpretation of experimental data applies very well in this work. By means of this method, data can be interpreted in terms of a much larger number of animals than those actually used. This statistical method is based on 2 assumptions: (1) an animal which dies from a given dose would

method, the LD_{50} for reference aconitine as shown in Table III was found to be 0.000399 mg./Gm. body weight of mouse, in terms of 695 mice when 104 mice were actually used. The result obtained in the determination of the LD_{50} for reference aconitine in terms of 28 mice when only 12 mice were actually used was found to be 0.000400 mg./Gm. body weight

TABLE III.—INTEGRATION OF REFERENCE ACONITINE LD_{50}[a] DETERMINATIONS

Dose in Mg./Gm. Body Weight	Experimental Data			Integrated Data		
	Alive	Dead	Per Cent Mortality	Alive	Dead	Per Cent Mortality
0.000220	1	1	50	55	1	1.78
0.000257	1	0	0	54	1	1.81
0.000265	1	0	0	53	1	1.85
0.000286	1	0	0	52	1	1.89
0.000300	6	1	14	51	2	3.77
0.000348	1	0	0	45	2	4.25
0.000350	9	5	35.7	44	7	13.70
0.000353	0	1	100	35	8	18.50
0.000375	9	7	43.7	35	15	30.00
0.000400	17	12	41.3	26	27	50.90
0.000405	1	1	50.0	9	28	75.60
0.000425	5	9	64.3	8	37	82.20
0.000450	3	9	75.0	3	46	93.80
0.000500	0	3	100.0	0	49	100.00
Totals	55	49		470	225	

[a] LD_{50} = 0.000399 mg./Gm. body weight of mouse.

TABLE IV.—INTEGRATION OF REFERENCE ACONITINE LD_{50}[a] DETERMINATION
(Bioassay, November 25, 1942)

Dose in Mg./Gm. Body Weight	Experimental Data			Integrated Data		
	Alive	Dead	Per Cent Mortality	Alive	Dead	Per Cent Mortality
0.000350	2	0	0	6	0	0
0.000375	1	1	50	4	1	20
0.000400	2	2	50	3	3	50
0.000425	1	1	50	1	4	80
0.000450	0	2	100	0	6	100
Totals	6	6		14	14	

[a] LD_{50} = 0.000400 mg./Gm. body weight of mouse.

of mouse as shown in Table IV. It is apparent that reliable results can be obtained using a small number, such as 12 mice, by applying this statistical method of interpretation of data.

In determining the optimum period of observation, the number injected animals dead were counted at the end of 3, 6, 12, 18 and 24 hr. after injection. Animals died from the effects of aconitine as long as 18 hr. after injection, but all those living after 24 hr., survived. Furthermore this 24-hr. period of observation provides a more efficient performance of the bioassay. The mice can be injected during the morning and the results read the next morning, instead of requiring the return of the operator to the laboratory at some odd and inconvenient time after laboratory hours. The size of the animal lends itself to simplicity in the performance of the assay in that one operator can easily hold and inject the animal at the same time.

CONCLUSIONS

1. The use of mice in the bioassay of tincture of Aconite has been found to yield accurate and uniform results. The standard error of each series of assays was found to be approximately 1%.

2. The use of mice produces a very economical and simple means of performing a bioassay of tincture of Aconite. Mice are an inexpensive laboratory animal to establish and maintain.

3. The use of the double integration method of statistical interpretation of data makes it possible to obtain accurate results using as small a number as 12 mice for each LD_{50} determination.

REFERENCES

(1) Rowe, L. W., THIS JOURNAL, 14 (1925), 968.
(2) Wright, H. N., *Ibid.*, 30 (1941), 177.
(3) Dille, James M., "Principles and Practice of Biological Assay" 3rd revision, Seattle, Washington: Department of Pharmacology, University of Washington, 1940, p. 31.
(4) Munch, James C., "Bioassays, A Handbook of Quantitative Pharmacology," Williams and Wilkins Co., Baltimore, Md., 1931, p. 30.
(5) *Ibid.*, p. 32.
(6) *Ibid.*, p. 35.
(7) "The Pharmacopœia of the United States of America," Twelfth revision, Mack Printing Co., Easton, Pa., 1942, p. 511.

A Study of the Microbiologic Assay of Riboflavin*,†

By R. D. Greene and Archie Black

Following the appearance of the Snell and Strong (1) microbiological assay method for riboflavin, we applied it to a number of pharmaceutical B complex products. While many assays were apparently satisfactory, the frequency of irregular standard curves and discordant results suggested the need for further study of the method. It appeared possible that the basal medium was incomplete and the introduction of certain modifications resulted in greater regularity and sensitivity in the response of *Lactobacillus casei* to riboflavin.

Several workers (2, 3, 4, 5, 6) have reported abnormal results from the microbiologic riboflavin assay of products such as blood and cereals which contain relatively low amounts of this vitamin. It has been found (4, 7) that such products contain lipoidal substances which stimulate the growth of *L. casei* when present in small amounts but may inhibit the growth when present in larger quantities. It has been shown (6, 8) that the removal of these substances is a necessary preliminary to the assay of crude natural products which are low in riboflavin.

In the assay of various types of B complex products of higher riboflavin content the modified procedure developed in this laboratory has yielded highly satisfactory results without special treatment of samples to remove interfering substances. It is believed that a description of the modified method and a comparison of some of the assays with concurrent biologic data may

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be of interest to others engaged in the assay of riboflavin.

EXPERIMENTAL

The original Snell and Strong (1) method for testing riboflavin frequently gave discordant results and a few typical data are contained in Table I. Extensive studies on the basal medium have been made and the more pertinent of these are described below.

Source of B Complex.—In examining the components of the Snell and Strong basal medium it appeared that the yeast supplement might be suboptimal in certain essential factors. In our search for an improved source of B complex, we tried replacing the lead-treated yeast supplement by others prepared by photolysis and by charcoal treatment (9) or by a mixture of synthetic vitamins similar to that reported by Silber and Unna (10). The photolyzed Difco yeast extract produced a higher response (Fig. 1) and has been used in an experimental medium (I) for the study of other factors. While this medium has not been used as extensively for assay as the modified assay medium (II) to be described later, consideration of curves 1 and 2 of Fig. 1 and actual assay data indicate that it may be suitable for this purpose.

Ammonium Salts, Additional Sodium Acetate.—It has been found that the growth of *L. casei* is stimulated by ammonium salts and additional sodium acetate (Fig. 1). Although the combined effect of these 2 salts is approximately duplicated by ammonium acetate, in practice a mixture of 2% ammonium sulfate and 2% additional sodium acetate has appeared preferable. A medium (II) containing photolyzed Difco yeast extract with these optimum levels of the 2 salts has been extensively used for assay purposes and has yielded high and regular responses to riboflavin, with negative controls that are satisfactorily low. Comparative assays by the Snell and Strong and modified methods are shown in Table I. It will be noted that the use of the modified medium permitted a considerable reduction in the levels of samples used for assay. This fact and the consequent decrease in any extraneous effects introduced may explain the increased concordance in the results.

Other Growth Factors.—It is believed of interest to report here on the studies of certain other factors which may affect the microbiological riboflavin assay or the growth of *L. casei* under other special conditions.

Clarke, Lechycka and Light (11), using a pantothenic acid assay medium (12), have reported that rice polishings concentrate contains an unidentified substance which greatly stimulated acid production by *L. casei*. Since this supplement appeared capable of affording stimulation both from B complex vitamins and fermentable carbohydrate, it seemed desirable to extend previous studies of these factors. With 100 mg. of glucose in 10 cc. of basal medium I, containing 40 mg. of photolyzed Difco yeast extract and 0.25–0.30 μg. of riboflavin,

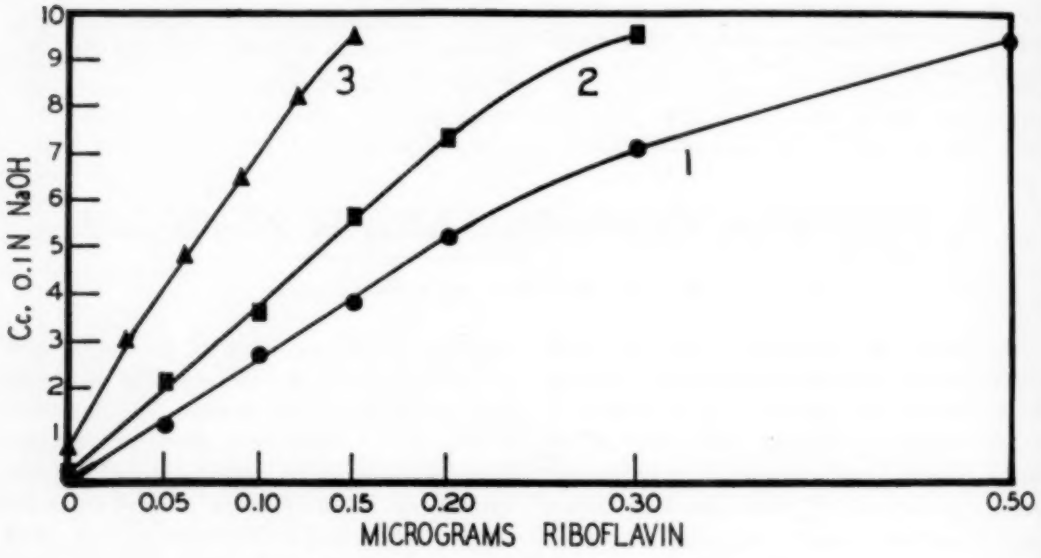

Fig. 1.—Standard Curves—Response to Riboflavin.
Curve 1, Snell and Strong medium.
Curve 2, Photolyzed Difco yeast extract medium (I).
Curve 3, No. 2 + 2.0% ammonium sulfate + 2.0% sodium acetate (II).

L. casei in 72 hr. usually produced 9–10 cc. of 0.1 *N* acid, and occasionally nearly up to the theoretical limit of about 11.0 cc. As shown in Table II, this amount of acid was not materially increased by separately raising the level of riboflavin or of glucose. However, when the level of glucose was doubled, increasing the riboflavin markedly stimulated acid production, which was further increased by the use of very high levels of synthetic B complex vitamins or Difco yeast extract. Acid production was also greatly increased by the addition of 400 mg. of rice polishings concentrate, but this supplement contained fermentable carbohydrate as shown by the production of 7.5 cc. of 0.1 *N* acid in the *absence* of glucose. It thus appears that under assay conditions the growth of *L. casei* in this riboflavin deficient medium is not limited by a deficiency of any unknown factor present in rice polishings concentrate.

Extended studies have been made of the effects of the addition to riboflavin deficient media of lipoidal supplements such as the free fatty acids. Since the work has, in general led to conclusions which confirm reports already published (2, 3, 4, 5, 6, 7, 8), it is not believed necessary to describe it here.

TABLE I.—MICROBIOLOGICAL ASSAYS BY THE SNELL AND STRONG AND MODIFIED METHODS

Sample No.	Product	Snell and Strong: Sample Level, Mg.	Snell and Strong: γ Riboflavin per Gm.	Modified[a]: Sample Level, Mg.	Modified[a]: γ Riboflavin per Gm.
2099	Dried Brewers' Yeast I	1.0	70.0	1.0	50.0
		2.0	56.0	1.5	50.0
		3.0	54.0	2.0	55.0
2216	Dried Brewers' Yeast II	1.0	92.5	0.5	80.0
		2.0	80.0	1.0	74.0
		3.0	97.5	1.5	81.0
2303	Dried Brewers' Yeast III	1.0	65.0	1.0	73.0
		2.0	80.0	1.5	86.5
		3.0	95.0	2.0	85.5
2245	Yeast Extract[b]	0.4	230.0	0.2	196.0
		0.6	270.0	0.3	188.0
2538	Yeast Extract[b]	0.4	225.0	0.2	198.0
		0.6	246.0	0.3	202.0
		0.8	280.0	0.4	203.0
2050	Rice Bran Extract[b]	10.0	10.8	5.0	10.8
		15.0	9.3	7.5	10.9
		20.0	11.2	10.0	9.9
2125	Wheat Germ Extract[b]	10.0	7.0	5.0	6.4
		15.0	6.0	7.5	6.3
		20.0	5.5	10.0	6.2

[a] Basal Medium II.
[b] Water-soluble market products.

TABLE II.—EFFECT OF GLUCOSE AND B COMPLEX SUPPLEMENTS ON ACID PRODUCTION

Expt.	Additions per 10 Cc. of Basal Medium I	Titration (Cc. 0.1 *N* NaOH): Control	Titration (Cc. 0.1 *N* NaOH): +100 Mg. Glucose
A 1	None	0.33	0.63
2	Riboflavin, 0.05 γ	2.30	2.50
3	Riboflavin, 0.10 γ	3.70	4.00
4	Riboflavin, 0.20 γ	6.74	6.76
5	Riboflavin, 0.30 γ	9.47	9.77
6	Riboflavin, 0.50 γ	9.71	12.50
B 1	Riboflavin, 1.00 γ	9.05	16.10
2	B1 + 20 γ each of riboflavin, pyridoxine, pantothenic acid, nicotinic acid	9.20	17.50
3	B1 + 300 mg. yeast extract (Difco)	8.70	17.50
4	B1 + 400 mg. rice polishing concentrate (Vitab)	16.58	...
5	B4 minus 100 mg. glucose	7.50	...

ASSAY PROCEDURE

The general procedure is substantially that of Snell and Strong (1), with certain modifications.

Basal Media.—Medium I (Experimental): Peptone-sodium acetate, cystine, glucose, inorganic salts A and B (1); photolyzed Difco yeast extract 0.4%. Medium II (Assay): Medium I + 2.0% ammonium sulfate + 2.0% sodium acetate.

Preparation of Photolyzed Yeast Extract.—A solution of 6 Gm. of Difco yeast extract in 150 cc. of distilled water is adjusted to *p*H 10.5 with sodium

hydroxide and photolyzed in a glass covered 10-in. crystallizing dish for 10 to 15 hr. Photolysis with 2 Mazda bulbs totaling 500 watts, at distances of 8 in. from the solution, with cooling to about 50° C. with a current of air, is effective. Other sources of light may be used; a 3000-watt carbon arc destroys the riboflavin in 1–2 hr. After neutralization with hydrochloric acid, the solution, equivalent to 40 mg. of yeast extract per cubic centimeter, is preserved under toluene.

Inoculation.—For culture of inoculum, a medium containing 0.1% photolyzed Difco yeast extract and 1.0 μg. of riboflavin in 10 cc., but otherwise identical with Medium I, was used. For inoculation, the more dilute inoculum referred to by Eckhardt, *et al.* (2) was used.

Preparation of Samples for Assay.—Samples other than water-soluble materials were autoclaved at a *p*H of about 5 with 100 volumes of water at 15 lb. pressure for 15 min. Insoluble material was removed by centrifugation or filtration.

MICROBIOLOGICAL AND BIOLOGICAL ASSAYS

Table III presents the microbiological and biological (13) assays of several types of pharmaceutical B complex materials of intermediate to high riboflavin potency. The assays reveal a fairly uniform relationship between the values obtained by the 2 methods, with the biological method yielding somewhat higher figures. It is possible that the biological method is inclined to give high results with natural B complex products because of some growth stimulation which is caused by factors other than riboflavin.

Table III.—Microbiological and Biological Assays for Riboflavin

Sample No.	γ Riboflavin per Gm. Microbiological[a]	Biological
	Dried Brewer's Yeast	
2242	73	100
2256	82	80
2303	81	100
2318	85	85
2362	86	100
2373	80	90
2391	79	100
2406	84	100
2415	85	75
2435	68	75
2444	70	100
2463	72	100
2514	75	85
2522	76	100
	Yeast Extract	
2245	192	215
2254	216	230
2263	210	260
2280	221	230
2294	206	270
2340	208	280
	Rice Bran Extract	
2162	11.6	10.8
2188	11.2	9.0
2224	11.0	11.6
2264	13.4	13.8
2287	13.0	14.0
2326	11.1	13.0
2336	11.4	14.6
2350	12.2	10.8
2426	9.0	12.3
	Riboflavin Concentrate	
2319	5500	5500
2330	5250	5400
2354	4700	5500
2368	4950	4800
2396	4550	5000
2418	4600	5200
2441	5150	5400
2547	5250	5000
2489	5050	5500

[a] Assays by the use of Basal Medium II.

SUMMARY

The effects of a number of factors of the growth of *L. casei* have been studied, and some of these have been utilized in the development of a modification of the Snell and Strong basal medium which increases the sensitivity and regularity of the response of the organism to riboflavin, with increased concordance in assay results. Comparative assays of numerous samples including yeast products and rice bran concentrates by this modified method and by a rat growth method are in fairly close agreement and indicate that this modified microbiological method is capable of giving reliable riboflavin assays of such products.

REFERENCES

(1) Snell, E. E., and Strong, F. M., *Ind. Eng. Chem., Anal. Ed.*, 11 (1939), 346.

(2) Eckhardt, R. E., Gyorgy, P., and Johnson, L. V., *Proc. Soc. Exptl. Biol. Med.*, 46 (1941), 405.

(3) Strong, F. M., Feeney, R. E., Moore, B., and Parsons, H. J., *J. Biol. Chem.*, 137 (1941), 363.

(4) Bauernfeind, J. C., and Sotier, A. L., Abs. 102d meeting A. C. S. (1941), B34.

(5) Scott, M. L., Randall, F. E., and Hessel, F. H., *J. Biol. Chem.*, 141 (1941), 325.

(6) Andrews, J. S., Boyd, H. M., and Terry, D. E., *Ind. Eng. Chem., Anal. Ed.*, 14 (1942), 271.

(7) Strong, F. M., and Feeney, R. E., *J. Biol. Chem.*, 142 (1942), 962.

(8) Strong, F. M., and Carpenter, L. E., *Ind. Eng. Chem., Anal. Ed.*, 14 (1942), 909.

(9) Strong, F. M., Feeney, R. E., and Earle, A., *Ibid.*, 13 (1941), 566.

(10) Silber, R. H., and Unna, K., *J. Biol. Chem.*, 142 (1942), 623.

(11) Clarke, M. F., Lechycka, M., and Light, A. E., *Ibid.*, 142 (1942), 957.

(12) Pennington, D., Snell, E. E., and Williams, R. J., *Ibid.*, 135 (1940), 213.

(13) Vitamin Assay Committee, *A. D. M. A. Proceedings* (1938, 1939).

U. S. Pharmacopœial Convention

Statement of Income and Expense for the Third Year of the 1940–1950 Decennial Period—May 1, 1942, to April 30, 1943

The accounts of the U. S. Pharmacopœial Convention for the year ending April 30, 1943, have been audited by the R. G. Rankin Company, New York and Washington. In conformity with action taken in previous years, the Board of Trustees has authorized the publication of the Statement of Income and Expense, and the Statement of Cash Receipts and Disbursements, as prepared by the auditors, together with the usual additional detail covering disbursements under the headings of Revision and Research.

The marked increase in the income of the Convention over the expense for the year is, of course, primarily found in the Sale of Pharmacopœias, most of which is obviously represented by the U. S. P. XII sales. The sale for the first few months for each new pharmacopœia is naturally extremely high, since a type of immediate saturation for the current industry is demanded. It is interesting to note that volume sales of U. S. P. XII almost parallel those of U. S. P. XI, U. S. P. XII sales leading, however, at all periods to date. It is obvious that the increased income during this initial period represents a reserve for the maintenance of the revision program for the coming years, a program which will finally bring forth another edition of the pharmacopœia, new, up-to-date and in keeping with the times.

The increased Publication and Sales expenses naturally represent, for the most part, costs of printing and binding for the U. S. P. XII to date. A portion of the initial printing is yet to be bound.

Administration, Convention, Revision, and Research costs have all shown a marked decrease for the year in practically every phase of expense. This might well be expected since the problems of the 1940 and 1942 Convention Meetings have, for the most part, been settled, and since a good portion of the year was taken with the final preparation of proof in its various forms, rather than with the more active and costly phases of revision. The preparation of text for the coming First U. S. P. XII Bound Supplement has, however, had its effect in the activities of the various branches. The continuous revision program which has now been adopted will undoubtedly mean increased activity during the coming months, looking to the revision of U. S. P. XII and the eventual appearance of U. S. P. XIII.

The repayment of Bank Loans is explained by the fact that for a short time previous to the appearance of the U. S. P. XII, the Board negotiated very satisfactory loans through its bank for current expenses, using some of its investments in government bonds as collateral. By its farsighted policy, the Board was thus able to have its extra cash invested during the several years following the appearance of U. S. P. XI, and through this recent transaction was able to keep these good investments intact. Had the Board maintained extra cash in reserve during all those years, considerable income would have been sacrificed, and less attractive investments would have been available for it today.

The usual additional breakdown of Revision and Research expenses follow.

For the U. S. P. Convention,
W. PAUL BRIGGS, *Treasurer*
For the U. S. P. Board of Trustees,
E. F. KELLY, *Chairman*
ADLEY B. NICHOLS, *Secretary*

U. S. PHARMACOPŒIAL CONVENTION, WASHINGTON, D. C.

STATEMENT OF INCOME AND EXPENSE FOR THE YEAR ENDED APRIL 30, 1943

Income				
Sale of Pharmacopœias:				
Collections			$167,256.10	
Add: Increase in Accounts Receivable:				
April 30, 1943		$27,412.48		
April 30, 1942		1,066.71	26,345.77	$193,601.87
Sale of Reference Standards, A. M. A. Articles, etc.:				
Collections			$ 6,125.25	
Less:				
Decrease in Collections Pending Remittance to the Treasurer				
April 30, 1942	$ 957.04			
April 30, 1943	274.50	682.54		
Decrease in Accounts Receivable				
April 30, 1942	$ 495.08			
April 30, 1943	405.59	89.49	772.03	5,353.22

Interest on Investments:				
Collections			$ 1,502.50	
Less: Amortization of Bond Premiums			270.05	1,232.45
Use of U. S. Pharmacopœia Text by Others				45.00
Miscellaneous Income				833.25
Total Income				$201,065.79
Less:				
Expense				
Cost of Publications Sold:				
Publication and Sales Expense		$40,241.83		
Less: Increase in Inventory				
April 30, 1943	$9,503.96			
April 30, 1942	4,228.75	5,275.21	$ 34,966.62	
Administration			3,399.01	
Convention			360.63	
Revision		$17,023.52		
Add: Decrease in Inventory of Reference Standards				
April 30, 1942	$3,691.26			
April 30, 1943	1,732.83	1,958.43	18,981.95	
Research			4,089.34	
Stationery and Supplies Inventory Adjustment			226.32	
			$ 62,023.87	
Less:				
Furnishings and Equipment Capitalized (at 75% of cost)			9.00	62,014.87
Excess of Income over Expense				
For the Year ended April 30, 1943				$139,050.92

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS (GENERAL ACCOUNT) FOR THE YEAR ENDED APRIL 30, 1943

Cash on Deposit, April 30, 1942		$ 16,414.60
Add:		
Receipts		
Sale of Pharmacopœias	$167,256.10	
Sale of Reference Standards, A. M. A. Articles, etc	6,125.25	
Interest on Investments	1,502.50	
Use of U. S. Pharmacopœia Text by Others	45.00	
Miscellaneous Income	833.25	175,762.10
		$192,176.70
Less:		
Disbursements		

	Publication and Sales	Administration	Convention	Revision	Research	Total
Printing & Binding	$40,076.69	$.....	$ 91.62	$.....	$.....	$40,168.31
General	165.14	559.14	.....	2,043.00	5.73	2,773.01
Clerical	.....	917.35	246.40	4,788.61	226.50	6,178.86
Meetings	.....	16.74	.....	998.78	880.41	1,895.93
Supplies	.....	23.25	13.03	1,194.59	546.56	1,777.43
Postage and Telegrams	.....	50.65	9.58	798.54	67.64	926.41
Honoraria & Salaries	.....	1,000.00	.....	7,200.00	.....	8,200.00
Compensation	.....	.....	.....	.....	2,362.50	2,362.50
Interest on Bank Loans	.....	831.88	.....	.....	.....	831.88
	$40,241.83	$3,399.01	$360.63	$17,023.52	$4,089.34	$65,114.33

Add: Repayment of Bank Loans	45,000.00	110,114.33
Cash on Deposit, April 30, 1943		$ 82,062.37

A MORE DETAILED AND SUPPLEMENTARY FINANCIAL STATEMENT OF THE REVISION EXPENSES MAY 1, 1942, TO APRIL 30, 1943

REVISION—MEETINGS

Traveling, hotel and incidental expenses of the Revision Chairman, Subcommittee Chairmen, Subcommittee and special committee groups, and for conferences with Food and Drug officials and others in maintaining the Revision program, and attendance of the Chairman at meetings of related organizations. Included in this figure are approximately 10 trips each to Washington and to New York, an Insulin conference at New York, numerous chemical conferences in Philadelphia preparing U. S. P. XII text, meetings of the A. D. M. A., the A. P. M. A., the American Hospital Association, a conference with the N. F. Committee at Pittsburgh and a Chicago Conference	$ 998.78

REVISION—SUPPLIES

Stationery	55.50
Mimeograph supplies (paper, ink, stencils, maintenance of machine)	368.46
Reference standards—new stock	497.94
Binders for circulars	84.00
Miscellaneous	188.69
	$1,194.59

REVISION—CLERICAL

Stenographic and clerical assistance, Chairman's office	4,788.61

REVISION—POSTAGE AND TELEGRAPH

Postage, Express, Telephone and Telegraph service, Chairman's office	798.54

REVISION—GENERAL EXPENSES

Translating "Pharmacopœia and Physician," Series II, into Spanish	96.00
Reprints—A. M. A. Articles, "Pharmacopœia and Physician," Series II	10.22
Contribution to the AMERICAN PHARMACEUTICAL ASSOCIATION toward Pharmaceutical Abstracts	1,000.00
Refrigeration and incidentals for maintenance of reference standards	99.05
A. M. A. Exhibit	224.52
Extending Information to Physicians and others through reprints and booklets	433.63
Inter-Society Color Council, dues for 2 years	50.00
Miscellaneous	129.58
	$2,043.00

REVISION—SALARIES AND HONORARIA

Salary, Revision Chairman, E. Fullerton Cook	4,800.00
Salary, Executive Assistant to Chairman, Adley B. Nichols	2,400.00
	$7,200.00

RESEARCH

Subcommittee		Meetings, Supplies, Postage, Clerical, Travel and General	Technical Assistance	
3—Biological Assays		$ 26.64		
5—Botany		9.04		
7—Inorganic Chemistry		52.96	Dr. F. K. Bell, Salary	$1,012.50
Supplies	32.96			
Clerical	10.00			
Post. & Tel.	10.00			
8—Organic Chemistry		750.00	Miss Eleanor Sackter, Salary	1,350.00
Supplies	294.75			
Meetings and Travel.	375.00			
Clerical	60.00			
Post. & Tel.	20.25			
Digitalis study and conferences		182.25		
Meetings	176.52			
Post. & Tel.	5.73			
Vitamin Advisory Board Meetings		296.38		
Anti-anemia Products Advisory Board.		234.57		
Supplies	43.85			
Meetings	32.51			
Clerical	141.00			
Post. & Tel.	17.21			
Suture test study apparatus		175.00		
		$1,726.84		$2,362.50
				1,726.84
Total				$4,089.34

Books Received

To July 1, 1943

L. Earle Arnow and Henry C. Reitz, *Introduction to Organic and Biological Chemistry.* The C. V. Mosby Company, St. Louis, Mo., 1943. 736 pp. $4.25.

H. Bennett, *Practical Emulsions.* Chemical Publishing Co., Inc., Brooklyn, New York, 1943. 462 pp. $5.00.

George A. Crossen and Karl J. Goldner, *Pharmaceutical Preparations.* The John S. Swift and Company, Inc., St. Louis, Mo., 1942. 167 pp.

Yandell Henderson and Howard W. Haggard, *Noxious Gases.* Second and revised edition. Reinhold Publishing Corporation, 330 West 42nd St., New York City, 1943. 294 pp. $3.50.

I. M. Kolthoff and V. A. Stenger, *Volumetric Analysis.* Vol. I, Second edition. Interscience Publishers, Inc., New York, 1942. 309 pp. $4.50.

Jack Neal Lott, Jr., and Robert Hanes Gray, *Law in Medical and Dental Practice.* The Foundation Press, Inc., Chicago, 1942. 499 pp. $4.75.

Rudolph Macy, *Organic Chemistry Simplified.* Chemical Publishing Co., Inc., Brooklyn, New York, 1943. 431 pp. $3.75.

William Marias Malisoff, *Dictionary of Bio-Chemistry.* Philosophical Library, Inc., 15 East 40th St., New York City, 1943. 579 pp. $7.50.

Maxim Newmark, *Dictionary of Science and Technology.* Philosophical Library, Inc., 15 East 40th St., New York City, 1943. 386 pp. $6.00.

John Ralph Nicholls, *Aids to the Analysis of Food and Drugs.* Bailliere, Tindall and Cox, London, 1942. 424 pp. $2.43.

Frank Welcher, *Chemical Solutions.* D. Van Nostrand Company, Inc., 250 Fourth Ave., New York City, 1942. 404 pp. $4.75.

Book Reviews

Nasal Medication, by NOAH D. FABRICANT, M.D., M.S., Williams and Wilkins Company, Baltimore, 1942. ix + 122 pp., 20 illus., 14 x 21.5 cm. Price, $2.50.

This book was written primarily for the physician and specialist, but pharmacists will find it to be a handy guide to modern nasal therapy. Of the 10 chapters in this book, 6 are given over to a discussion of nasal medication. The remaining chapters consider the anatomy, histology, histopathology and physiology of the nasal passages. This review will be confined to the chapters on medication.

The chapters of primary interest to the pharmacist are: The Effect of Drugs on Ciliary Action and Nasal *p*H, The Effect of Drugs on the Mucous Membrane, Methods of Applying Nasal Medication in Disease, and Nasal Medication in Children.

It is the thesis of Dr. Fabricant that modern nasal medication must take into account the action of the drug on the mucous membrane and ciliary action. Of particular concern in this interrelationship is the *p*H and the tonicity of the medicament. It is shown that many such commonly used drugs as 1:1000 epinephrine solutions actually interfere with ciliary activity, a factor that is even more detrimental in the presence of congestion.

A careful study of this fine review will aid the foreward-looking pharmacist in formulating the type of nasal medication that is of interest to the present-day physician.—M. W. GREEN.

Practical Survey of Chemistry and Metabolism of the Skin, by MORRIS MARKOWITZ, M.D., The Blakiston Company, Philadelphia, Pa., 1942. xii + 196 pp., 12.5 × 20 cm. Price $3.50.

The main purpose of this book is to furnish a general survey of the fundamental facts necessary for a better understanding of dermatology.

The book is divided into four parts. Part I discusses the chemistry of the skin. From the viewpoint of the chemist, this section leaves much to be desired. There are a number of errors of both omission and commission and several misconceptions. One wonders, for example, why the author insists on calling the element silicon, "silicum." In discussing zinc, no mention is made of the role of zinc in carbonic anhydrase. In a discussion on enzymes the author states, "They are produced by the cells of the salivary gland, the stomach, the intestines and the pancrease"—what a limitation for such ubiquitous substances. On the other hand, the chapters on acid-base balance and the absorptivity and permeability off the skin should be most helpful to the pharmacist.

Part II of this little manual is concerned with Hematology, while Part III discusses Blood Chemistry as it is applied to dermatology. This latter section, which is well documented, should be useful to the practicing dermatologist.

Part IV concerns the Vitamins in Dermatoses. This section is unfortunately short and inclined to be unimaginative.

The pharmacist and, more particularly, the pharmaceutical research worker with imagination will find this little book useful in determining some of the desired characteristics of good ointment formulation. The bibliography throughout is good and appears to be reasonably thorough and selective.—M. W. GREEN.